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

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Received 30 July 2007; accepted 24 July 2008
DOI 10.1002/app.29001
Published online 13 October 2008 in Wiley InterScience (www.interscience.wiley.com).

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 drying9,19 techniques are generally used for microencapsulation.

Varieties of crosslinking agents like glutaraldehyde, formaldehyde, epoxy compounds20–22 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.,
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 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, and stirring was continued for about 3-4 h in order to complete the crosslinking reaction. Once the coacervation took place with the formation of microcapsules, the system was brought to room temperature (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. soln). 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

\[
\text{Encapsulation efficiency} \% = \frac{w_1}{w_2} \times 100
\]

\[
\text{Oil content} \% = \frac{w_1}{w} \times 100
\]

\[
\text{Oil load} \% = \frac{w_3}{w_2} \times 100
\]

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.
Microencapsulation of Zanthoxylum limonella oil

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 (~ 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 the large 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>
<thead>
<tr>
<th>Oil load (%)</th>
<th>Oil content (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.00</td>
<td>26.45 ± 1.20</td>
<td>34.14 ± 1.74</td>
</tr>
<tr>
<td>6.06</td>
<td>25.15 ± 0.97</td>
<td>32.47 ± 1.07</td>
</tr>
<tr>
<td>6.10</td>
<td>30.65 ± 0.54</td>
<td>39.56 ± 1.74</td>
</tr>
<tr>
<td>6.15</td>
<td>37.65 ± 0.75</td>
<td>48.60 ± 1.14</td>
</tr>
<tr>
<td>6.20</td>
<td>42.32 ± 0.15</td>
<td>48.31 ± 0.84</td>
</tr>
<tr>
<td>6.25</td>
<td>42.00 ± 0.45</td>
<td>54.22 ± 1.12</td>
</tr>
<tr>
<td>6.30</td>
<td>45.34 ± 0.87</td>
<td>58.53 ± 1.36</td>
</tr>
<tr>
<td>6.35</td>
<td>46.50 ± 0.64</td>
<td>60.05 ± 0.89</td>
</tr>
</tbody>
</table>

Total polymer = 1 g; genipin = (0.05-0.5 mmol/gm of polymer; oil = (1-4 mL); water = 100 mL; Temperature = (40 ± 1)°C.
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\textsuperscript{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\textsuperscript{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...
with the increase in gencpin concentration. The concentration of gencpin 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 gencpin, gelatin, and chitosan. The release rate of oil was found to decrease as the % of gencpin 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.

Scanning electron microscopy study

Scanning electron microscopy (SEM) micrographs of gencpin 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. 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 whereas those prepared at low oil loading appeared dry and powdery.

**Thermogravimetric analysis**

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

<table>
<thead>
<tr>
<th>Sample particulars</th>
<th>Gelatin (gm)</th>
<th>Oil (ml)</th>
<th>( T_d ) (^{\circ} \text{C} )</th>
<th>RW (%) at 600 (^{\circ} \text{C} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>236</td>
<td>875</td>
</tr>
<tr>
<td></td>
<td>1 0.5</td>
<td>4.0</td>
<td>175</td>
<td>933</td>
</tr>
<tr>
<td></td>
<td>1 0.1</td>
<td>4.0</td>
<td>190</td>
<td>2306</td>
</tr>
<tr>
<td></td>
<td>0 0.1</td>
<td>-</td>
<td>273</td>
<td>37.74</td>
</tr>
<tr>
<td></td>
<td>- 0.01</td>
<td>- Oil(^{1})</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\( T_d \), initial decomposition temperature, RW, residual weight

\(^{1}\) Stated decomposing from the very beginning.

*Journal of Applied Polymer Science* DOI 10.1002/app
TABLE III
Temperature of Decomposition ($T_D$) at Different Weight Loss (%) of Virgin Polymer and Oil Containing Microcapsules

<table>
<thead>
<tr>
<th>Sample particulars</th>
<th>Temperature of decomposition ($T_D$) ($^\circ$C) at different weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin (gm) : Chitosan (gm)</td>
<td>OIL (mL)</td>
</tr>
<tr>
<td>1 : 0</td>
<td>–</td>
</tr>
<tr>
<td>1 : 0.5</td>
<td>4</td>
</tr>
<tr>
<td>1 : 1</td>
<td>4</td>
</tr>
<tr>
<td>0 : 1</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>OIL</td>
</tr>
</tbody>
</table>

Microcapsules. Both the Ti ($^\circ$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. 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
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\(^{-1}\) due to carbonyl stretching band. Besides this, the other notable peaks appeared at 1457 cm\(^{-1}\) and 1378 cm\(^{-1}\) due to \(\text{CH}_2\) asymmetric deformation and \(\text{CH}_2\) 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 \(\text{CH}_2\) 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), 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 disappear and a new peak appeared (at 120°C) when gelatin was used (cross-linked samples). The position of other two peaks in the thermogram of physical mixture remained almost unchanged irrespective of addition of gelatin. The peaks found at 240°C and 320°C in cross-linked 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 gelatin as cross-linker. 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 gelatin, 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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*Journal of Applied Polymer Science DOI 10 1002/app*
Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of Zanthoxylum limonella oil (ZLO) using salting-out method

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First Published: 2008


To link to this Article: DOI: 10.1080/02652040802025901
URL: http://dx.doi.org/10.1080/02652040802025901

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Preparation of genipin cross-linked chitosan-gelatin microcapsules for encapsulation of *Zanthoxylum limonella* oil (ZLO) using salting-out method

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(Received 27 August 2007; accepted 27 February 2008)

Abstract

*Zanthoxylum limonella* oil (ZLO) containing chitosan-gelatin complex microcapsules cross-linked with genipin, a cross-linker of natural origin, have been prepared by a complex coacervation process using the salting-out method. The effects of various parameters such as oil loading, degree of cross-linking, ratio of chitosan to gelatin, etc. on oil content, encapsulation efficiency and the release rate of ZLO have been studied. FT-IR spectroscopy has been used to understand the interaction between the polymers and oil. Scanning electron microscopy (SEM) has been employed to study the morphology of the prepared microcapsules.

Keywords: Chitosan, gelatin, ZLO, genipin, release studies

Introduction

For many chemists, an effective alternative to DEET (*N*, *N*-diethyl-m-toluamide) for personal protection against mosquitoes and biting flies is the holy grail (Isman 2006). In spite of five decades of research, no chemical has been found that provides the degree of protection against biting mosquitoes or persistence on human skin afforded by DEET. Concerns with the safety of DEET, especially to children (Fradin 1998), have resulted in the introduction of several plant oils as natural alternatives.

Plant essential oils, commonly used as fragrances and flavouring agents for foods and beverages, were recommended as an alternative source for insect control (Isman. 1999). Essential oil derived compounds can be applied to humans in a similar way to other conventional insecticides and they have little or no harmful effects (Hadfield-Law 2000, Mumcuoglu et al. 2002). The promising essential oils with repellent activity are derived from a large number of plants including *Cymbopogon* spp. (Ansari and Razdan 1995), *Mentha Piperita* (Ansari et al. 2000), *Ocimum* spp. (Tawatsin et al. 2001), *Zanthoxylum Limonella* (Das et al. 2003), Osage orange and catnip (Peterson and Coats 2001), *Zanthoxylum piperitum* (Pitasawat et al. 2007) and *Eucalyptus maculata* citriod (Collins and Brady 1993). However, the repellency of these essential oils is commonly lower in both efficacy and duration than that of synthetic repellents, principally DEET. Furthermore, essential oils undergo environmental deterioration by heat, humidity, light and oxygen (Edris and Bergnstahl 2001).

Microencapsulation of essential oils by polymeric materials is expected to slow down the release of essential oils and guarantee more protection against atmospheric conditions, thus providing them a longer shelf-life (Rosenberg et al. 1990, Maji et al. 2007). Another reason for microencapsulation is that the process enables conversion of liquid ingredients into fine powders—products with new properties.

Microencapsulation is a technique whereby liquid droplets or solid particles are packed into continuous individual shells. The shells or walls as they are called are designed to protect the encapsulated material (core) from factors that may cause deterioration. By a different approach, the wall is designed to permit...
controlled release of the encapsulated material (core) under desired conditions.

The microcapsule wall material can be formulated by using a wide variety of materials including natural and synthetic polymers. The selection of the microcapsule wall material is of utmost importance, as it constitutes the main critical point for providing stability and cost efficiency to the system and must be compatible with the microencapsulating technique followed (Pedroza-Islas et al. 2002). Furthermore, the wall material must release the encapsulated material into the final product during the application.

Numerous techniques for microencapsulation for various core materials have been reported in the literature. Out of the various techniques available in the literature, the coacervation process (both complex and simple coacervation) for encapsulation has been extensively used. This study focused on coacervation which is based on a controlled phase separation induced by macromolecule desolvation. If two oppositely charged polyelectrolytes are simultaneously involved, the process is called complex coacervation (Bodmeier et al. 1996, Palmieri et al. 1996, Kim et al. 2001, Peniche et al. 2003; Tammisetti and Thimma 2003, Xing et al. 2004) and in simple coacervation the desolvation of one polymer is induced by salt addition, pH, temperature modification, etc. (Bachtis and Kipperisides 1996, Mauguet et al. 2002, Maji et al. 2007). In complex coacervation, electrostatic interactions between two oppositely charged polycations play the major role.

Chitosan is a hydrophilic, biodegradable and biocompatible positively charged polysaccharide of low toxicity, which in recent years has found applications in cosmetic, biotechnology and drug delivery systems. This polymer has mucoidhesive properties due to its positive charges at neutral pH that enable an electrostatic interaction with mucous or a negatively charged mucosal surface. On the other hand, gelatin is an abundant protein and it is derived from collagen. There are two types of gelatin, namely Type A and Type B. Type A is positively charged below pH 8.0 and type B is negatively charged above pH 5.0. Gelatin is surface active and it is used as an emulsifier for various oils. Thus, gelatin is able to be an ideal candidate for the wall material of microcapsules. Again, in order to improve the controlled release behaviour, varieties of cross-linking agents are used. Most of the cross-linking agents are synthetic and not free from problems caused by physiological toxicity. Genipin, a natural cross-linker, whose cytotoxicity, feasibility and biocompatibility have well been studied are reported (Sung et al. 1999, Mi et al. 2000). It is 10 000 times less toxic than glutaraldehyde (Sung et al. 1999). In the present investigation, a modified complex coacervation method was used to produce zanthoxylum limonella oil (ZLO) containing chitosan-gelatin microcapsules cross-linked by genipin, a natural cross-linking agent. To the best of the authors' knowledge, genipin cross-linked gelatin-chitosan microcapsules containing ZLO by using salting-out technique has not been investigated to date. This communication prepares and reports the effect of different parameters on release characteristics of oil.

Materials and methods

Materials

Gelatin (G) type B (from bovine skin, 225 bloom) and chitosan (C) (medium molecular weight, viscosity 200 cps) for microcapsule wall material were purchased from Sigma-Aldrich Inc. (USA). Anhydrous sodium sulphate, Tween 80, acetic acid and sodium hydroxide were obtained from E. Merck (India). Essential oil ZLO, the core material, was extracted and purified in the laboratory and used. The Genipin (Mw=226.23) was purchased from Challenge Bioproducts Co. (Taiichung, Taiwan). All other reagents and solvents used were of analytical grade.

Coacervation behaviour study

The study of phase separation behaviour of aqueous solution of chitosan-gelatin mixture in the presence of sodium sulphate solution is required in order to optimize the coacervation process. The coacervation process depends on several factors like polymer-to-salt ratio, temperature, etc.

Aqueous solution (0.025% w/v) of chitosan in 1% (v/v) acetic acid and 0.04% (w/v) aqueous solution of gelatin in deionized water were prepared. The solution of chitosan and gelatin were mixed at different ratios (1:0.50–2.0) at room temperature (~30°C) under stirring condition. Now a predetermined amount of aqueous sodium sulphate solution (20% w/v) was added to each polymer mixture containing chitosan and gelatin at different ratios at room temperature. The ratio of total polymer-to-sodium sulphate was varied from 1:2 to 1:30. The temperature was varied from 30–50°C. The minimum temperature and polymer-salt ratio at which clear phase separation occurred were recorded.

Preparation of microcapsules

Chitosan flakes (2.50 g) were dissolved in 100 ml of 1% (w/v) acetic acid solution by stirring overnight in a conical flask until a clear solution was obtained. Gelatin solution was prepared by swelling 4.0 g of gelatin in (type B) in 100 ml double distilled cold water followed by heating until the appearance of a clear solution. Variable amounts of gelatin and chitosan solution were taken in a beaker at room temperature (~30°C) so that the weight ratios of chitosan to gelatin were 0/1, 0.33/0.67, 0.5/0.5, 0.67/0.33 and 1/0. To this mixture, a drop of silicon anti-foaming agent and essential oil (1–8 ml) were added under high agitation
by mechanical stirring to form an emulsion. Initially the coacervation of chitosan was brought about by gradual addition of aqueous sodium sulphate solution (20\% w/v) for ~2-2.5 h. In this stage ZLO encapsulated chitosan particles/microcapsules were formed. The pH of the entire mass of the beaker was then brought between 7.0-8.0 to induce interaction between chitosan, gelatin (type B) and genipin. The cross-linking of the chitosan-gelatin microcapsule was achieved by addition of a certain amount of aqueous genipin solution (0.1-0.3 mmol g\(^{-1}\) of polymer). The temperature of the vessel was maintained between 40-50\(^\circ\)C and stirring was continued for another 3 h. The vessel was then cooled to room temperature. The microcapsules were filtered, washed initially with 0.3\% Tween 80 solution to remove excess oil adhered to the surface and finally with double distilled water, dried and kept in a storage vial.

**Measurements**

A known concentration of essential oil in distilled water containing 0.3\% Tween 80 was scanned in the range of 200-400 nm by using a UV-visible spectrophotometer. For ZLO having concentration in the range 0.005-0.10 gm/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 were crushed using a mortar and carefully taken in a volumetric flask containing a known amount of 0.3\% aqueous Tween 80 solution and kept for sufficient time under 'continuous stirring condition for ensuring complete extraction of oil from the microcapsules. The encapsulation efficiency (\%), oil content (%) and oil loading (%) were calculated by using the calibration curve and the following formulae:

\[
\text{Encapsulation efficiency (\%) = } \frac{W_1}{W_2} \times 100
\]

\[
\text{Oil content (\%) = } \frac{W_1}{W} \times 100
\]

\[
\text{Oil load (\%) = } \frac{W_2}{W_3} \times 100
\]

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 cross-linker. Each measurement was performed in triplicate and average ±SD value was taken.

**Oil release studies**

Oil release studies of encapsulated oil were evaluated using a UV-visible spectrophotometer (UV-2001 Hitachi). A known quantity of microcapsules was placed directly into a known volume of 0.3\% Tween 80 surfactant solution. The microcapsule—Tween 80 mixture was magnetically stirred at a constant rate and the temperature throughout was maintained at 30\(^\circ\)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\% Tween 80 solution was returned to the container.

**Fourier transform infrared measurements**

FT-IR spectral measurements were performed using a Nicolet (model impact-410, USA) spectrophotometer. Microcapsules were grounded finely with KBr and FT-IR spectra were taken in the range of 400-4000 cm\(^{-1}\).

**Scanning electron microscopy**

Dry microcapsules were mounted on a metal stubs, sputtered with gold and viewed in a scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 15 kV and at room temperature.

**Results and discussion**

**Phase separation behaviour**

The phase separation behaviour of chitosan and gelatin (type B) were studied at first in order to get an idea regarding minimum temperature or polymer–salt ratio to be required individually. Gelatin (type B) solution did not produce any coacervate at any sodium sulphate concentration and temperature, whereas chitosan produced coacervate throughout the temperature range and salt ratio studied. In the case of the mixture of chitosan–gelatin, the minimum ratio of polymer mixture to salt and temperature at which clear phase separation observed were 1: 5 and 40\(^\circ\)C, respectively. Therefore, all the experiments for microencapsulation were done by maintaining the ratio of polymer-to-salt and temperature at 1 : 5 and 40\(^\circ\)C, respectively.

**Effect of variation of oil loading.**

The effect of variation of oil loading on the encapsulation efficiency, oil content and release rate is shown in Table I and Figure 1. With the increase of oil loading, the release rate of the oil from the chitosan gelatin microcapsules increased throughout the range of oil
The effect of variation of cross-linker concentration

Table 1. Effect of variation of oil loading, chitosan-to-gelatin ratio, and genipin concentration on the behaviour of microcapsules (Chitosan/gelatin mixture: 1 gm; Genipin: 0.1–0.3 mmol gm\(^{-1}\) of mixture, oil: 1–8 ml; water: 100 ml; temperature: 40±1°C).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chitosan/gelatin (C/G)</th>
<th>Genipin</th>
<th>Oil load (%)</th>
<th>Oil content (%) (M ± SD)</th>
<th>Encapsulation efficiency (%) (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0/1.0*</td>
<td>0.1</td>
<td>4.0</td>
<td>343.67</td>
<td>42.20 ± 1.67</td>
<td>48.36 ± 1.52</td>
</tr>
<tr>
<td>0.33/0.67</td>
<td>0.1</td>
<td>4.0</td>
<td>343.67</td>
<td>37.59 ± 0.07</td>
<td>48.53 ± 1.00</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.1</td>
<td>4.0</td>
<td>343.67</td>
<td>46.25 ± 2.36</td>
<td>52.65 ± 2.38</td>
</tr>
<tr>
<td>0.67/0.33</td>
<td>0.1</td>
<td>4.0</td>
<td>343.67</td>
<td>62.60 ± 2.10</td>
<td>80.80 ± 1.20</td>
</tr>
<tr>
<td>1.0/0.0</td>
<td>0.1</td>
<td>4.0</td>
<td>343.67</td>
<td>39.78 ± 1.52</td>
<td>51.36 ± 1.96</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.3</td>
<td>4.0</td>
<td>343.67</td>
<td>51.30 ± 1.02</td>
<td>66.22 ± 1.31</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.1</td>
<td>1.0</td>
<td>85.92</td>
<td>25.17 ± 0.34</td>
<td>54.46 ± 0.75</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.1</td>
<td>2.0</td>
<td>171.83</td>
<td>23.80 ± 1.01</td>
<td>40.82 ± 1.60</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.1</td>
<td>6.0</td>
<td>515.50</td>
<td>39.92 ± 0.20</td>
<td>47.67 ± 0.24</td>
</tr>
<tr>
<td>0.50/0.50</td>
<td>0.1</td>
<td>8.0</td>
<td>687.34</td>
<td>45.06 ± 3.03</td>
<td>51.60 ± 3.47</td>
</tr>
</tbody>
</table>

*Gelatin (type B) produced no coacervation.

Figures 1. Effect of variation of oil loading on the release rate. (a) CG (0.5/0.5), ZLO 1.0 ml; Gp 0.1 mmol; (b) CG (0.5/0.5), ZLO 2.0 ml, Gp 0.1 mmol; (c) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.1 mmol; (d) CG (0.5/0.5), ZLO 6.0 ml, Gp 0.1 mmol; (e) CG (0.5/0.5), ZLO 8.0 ml, Gp 0.1 mmol.

The effect of variation of cross-linker concentration on encapsulation efficiency (%), oil content (%) and stirrer became less efficient and larger oil vesicles were produced as a result. Also there was an increased tendency for the oil vesicles to coalesce at higher oil loads, so that the larger oil vesicles were formed and more oil could be encapsulated with the same amount of encapsulating material at the expense of decrease of thickness of microcapsule wall. At this time, the amount of polymer might not be sufficient for encapsulation of all oil vesicles. The chances of existing of some oil vesicles without encapsulation became more. The loss of these oil vesicles during isolation might cause a reduction in encapsulation efficiency. Both thickness of the wall and extent of cross-linking govern the release rate. In all these cases, it was assumed that the level of cross-linking remained almost same. Therefore, the thickness of the wall might play a predominant role for controlling the release rate. The increase in the oil load would result in a decrease of wall thickness, as explained earlier. The faster release rate of the microcapsule at higher loading might be due to decreased wall thickness of the microcapsule. With the decrease in wall thickness of the microcapsule, diffusional pathway for the oil release became short (Madan 1981, Senjokovic and Jalsenjak 1981) which resulted in an increase of release rate. Similar type of results was reported in the literature (Maji et al. 2007). Again with the increase in percentage oil load, the oil content (%) increased. At low oil load, many of the microcapsules have few or no oil vesicles in them, indicating that there was an abundance of the encapsulating polymer for the oil present. When the amount of the used oil increased, there was an increase in the number of oil vesicles in the microcapsules which resulted in an increase of oil content.
Figure 2. Scanning electron micrographs of microcapsules prepared with oil load (%) (a) 343.67 and (b) 687.34.

Figure 3. Effect of variation of cross-linker on the release rate. (a) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.1 mmol; (b) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.2 mmol; (c) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.3 mmol.

The related results are shown in Table I and Figure 4, respectively. The results found were as per expectation. The increased encapsulation efficiency (%) might be due to the improvement of oil retention capacity of the microcapsule caused by the reaction between cross-linking agent genipin and microcapsule wall material, chitosan and gelatin. An increase in the degree of cross-linking as expressed by molar concentration of genipin used, resulted in a decrease in oil release rate throughout the genipin concentration studied (0.1 mmol per gram of polymer-0.5 mmol per gram of polymer). As the degree of cross-linking of microcapsule wall material increased, the microcapsule wall became denser, resulting in the decrease of diffusion rate of the oil through the microcapsule wall. Similar type of observations were reported in the literature (Bachtsi and Kipparissides 1996, Maji et al. 2007). The probable reactions between gelatin, chitosan and genipin are presented in Figure 3.

Effect of variation of chitosan-to-gelatin ratio

The related results are shown in Table I and Figure 4. The release rate of oil from gelatin-chitosan microcapsules was dependent on the percentage of chitosan present in the mixture. The higher the percentage of chitosan in the chitosan-gelatin mixture, the lower was the release rate. The lower release rate might be due to the formation of microcapsules having more compact wall. It was known that every glucosamine unit of chitosan could react with genipin, whereas only primary amine groups of lysine and arginine residues on gelatin could react with genipin. The lysine and arginine residues in gelatin are much less. On the other hand, chitosan has a more average number of primary amine groups than gelatin for the reaction with genipin (Mi 2005). The more the percentage of chitosan in the chitosan-gelatin mixture, the higher the reaction between chitosan and genipin. As a result, more cross-linking would take place. This would in turn form a more compact wall, resulting in a decrease of release rate. A decrease in the release rate was reported (Kim et al. 2006) in the literature during studying of release behaviour of triclosan encapsulated in chitosan-gelatin microcapsules.

FTIR study

Figure 5 shows the FTIR spectra of gelatin, chitosan, ZLO and ZLO containing chitosan-gelatin microcapsules. The spectrum of ZLO displayed peaks around 1673 and 1720 cm⁻¹ which were due to carbonyl stretching band. The other peaks, which appeared at 1463 and 1383 cm⁻¹, were due to CH₂ asymmetric and CH₂ symmetric deformation. The spectrum of chitosan showed an amide characteristic peak at 1633 cm⁻¹. Gelatin was characterized by its carbonyl peak and amino band appeared at 1624 and 1547 cm⁻¹, respectively. The shifting of carbonyl band to 1641 cm⁻¹ indicated an interaction between gelatin and chitosan. The position of this peak did not alter when compared to that of spectrum of ZLO. The position of other peaks also were found to remain unchanged. This suggested that there was no significant interaction between chitosan-gelatin complex and ZLO.

Scanning electron microscopy study

Figure 6 shows the SEM photographs of microcapsules having different percentages of oil loading. At higher oil loading (Figure 6(b)), a bursting look was observed and it appeared more compared to those of microcapsules
Preparation of genipin cross-linked chitosan-gelatin microcapsules

Figure 4 Reaction scheme between chitosan, gelatin and genipin

Figure 5 Effect of variation of chitosan-to-gelatin ratio on release rate (a) CG (0/1), ZLO 4.0 ml, Gp 0.1 mmol, (b) CG (0.33/0.67), ZLO 4.0 ml, Gp 0.1 mmol, (c) CG (0.5/0.5), ZLO 4.0 ml, Gp 0.1 mmol, (d) CG (0.67/0.33), ZLO 4.0 ml, Gp 0.1 mmol, (e) CG (1/0), ZLO 4.0 ml, Gp 0.1 mmol

prepared at low oil load (Figure 6(a)). Moreover, on physical examination, the surface of the microcapsules containing higher percentages of oil appeared more oily and agglomerated compared to those of microcapsules containing lower percentages of oil.

Conclusion

The study showed that the entrapment of ZLO into chitosan-gelatin microcapsules could be achieved using the salting-out procedure. The release of ZLO was found to be dependent on percentage of oil loading, cross-linking density and chitosan-gelatin ratio. There was an interaction between chitosan and gelatin during the formation of complex, as evident by FTIR study. The study also showed no significant interaction between oil and chitosan-gelatin matrix.
SEM study showed the presence of oil on the microcapsule surface.

Acknowledgement

Financial support from Defence Research Laboratory, Tezpur is gratefully acknowledged.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figure 6 FTIR spectra of (a) gelatin B, (b) chitosan, (c) oil, and (d) oil containing microcapsules

The authors alone are responsible for the content and writing of the paper.
Microencapsulation of *Zanthoxylum limonella* oil (ZLO) in glutaraldehyde crosslinked gelatin for mosquito repellent application

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Received 16 November 2005; received in revised form 7 March 2006; accepted 19 March 2006
Available online 11 May 2006

Abstract

Glutaraldehyde (GA) crosslinked gelatin (G) microcapsules containing *Zanthoxylum limonella* oil (ZLO) were prepared by coacervation technique. The effect of various parameters such as variation of oil-loading, gelatin concentration and degree of crosslinking on release rate of oil were studied. Scanning electron microscopy (SEM) was used to understand the surface characteristics of microcapsules. FTIR-results indicated the absence of any significant interaction between polymer and oil.

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Keywords: Microencapsulation; Essential oil; Crosslinker; Gelatin

1. Introduction

Use of essential oil is the subject matter of many investigations for in recent years due to its eco-friendly and biodegradable nature. Various essential oils used as mosquito repellent have been reported in the literature (Sukumar et al., 1991; Sharma et al., 1993; Quarles, 1996; Brown and Hebert, 1997; Ansari et al., 2000; Tawatsin et al., 2001; Prajapati et al., 2005). Volatile oil of *Zanthoxylum hamiltonianum* (timur) has been found to posses effective mosquito larvicidal properties (Nath et al., 1989).

Controlled release formulation seems to be the best choice for increasing the efficiency and minimization of environmental damage. Out of various techniques available in the literature, coacervation process for encapsulation has been extensively used. Gelatin (Rosenblat et al., 1989), polyvinyl alcohol (Bachtsi and Kipparissides, 1996) and various other polymers (Salib et al., 1986; Beyger and Nairn, 1986) have been employed for the production of microcapsules. The permeation characteristic of coacervated crosslinked gelatin-acacia membranes to various active agents (Jalsenjak and Kondo, 1981; Nixon and Wang, 1989) have been reported in the literature. The release characteristics of ZLO in crosslinked gelatin have not been investigated to the best of our knowledge. The present investigation aimed at to study the release characteristic of oil containing microcapsules prepared under different conditions.

2. Methods

2.1. Materials

Gelatin (E. Merck, India), glutaraldehyde 25% w/v (E. Merck, Germany), anhydrous sodium sulphate (E. Merck, India), Tween 80 (S.d. fine chemicals, Mumbai) were used as received, without further purification. Essential oil (ZLO) was obtained from *Zanthoxylum limonella* plant as per description in the extraction section. Besides this, other reagents used were of analytical grade.

2.2. Essential oil extraction

Seeds of *Z. limonella* a big tree available in the Solmara area of Tezpur, were collected and shed dried for three to
four days. Essential oil was obtained by steam distillation of the seeds. The oil obtained was separated from aqueous phase and dried by treatment with anhydrous sodium sulphate. The dried oil was transferred into a dark glass bottle and kept at 4°C for subsequent use.

2.3. Phase separation behaviour of gelatin

A series of experiments were carried out to determine the cloud point temperature of a gelatin solution as a function of sodium sulphate concentration. Flask containing a certain amount of gelatin was immersed in a thermostatic water bath maintained at 5°C. A predetermined amount of aqueous sodium sulphate solution (10%, w/v) was added to the flask under stirring condition and the temperature of the water bath was gradually raised. The temperature at which the onset of phase separation started was recorded.

2.4. Encapsulation procedure

In a reaction vessel, 4–10% (w/v) of an aqueous solution (50 ml) of gelatin was taken at 30°C. To this, essential oil (3–15 ml) was added under high agitation to form an emulsion. The temperature of the vessel was then raised to 40°C. Coacervation was brought by gradual addition of aqueous sodium sulphate solution (20% w/v) for about 90 min. The vessel was kept at this temperature for another 30 min. The temperature of the vessel was then brought down to about 5°C. The crosslinking of the polymer capsule was achieved by slow addition of certain amount of glutaraldehyde (1–10 mmol/g of gelatin) solution, which consisted of methanol 16.67%, acetic acid 5%, sulphuric acid 0.17% and glutaraldehyde 25%. The temperature of the vessel was then raised to 40°C and stirring was continued for about 3–4 h. The vessel was cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 solution, dried and stored in a glass bottle.

2.5. Measurements

A known concentration of essential oil in distilled water containing 0.3% Tween 80 was scanned in the range of 200–400 nm by using UV visible spectrophotometer. For ZL0 having concentration in the range 0.005–0.1 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 ZL0 was obtained by knowing the absorbance value.

2.5.1. Encapsulation efficiency, oil content and oil load

A known amount of accurately weighed crushed microcapsules was taken in a volumetric flask containing a known amount of 0.3% aqueous Tween 80 solution and kept overnight with continuous stirring. The encapsulation efficiency (%), oil content (%) and oil loading (%) were calculated by using the calibration curve and the following formulae:

Encapsulation efficiency (%) = \( \frac{w_1}{w_2} \times 100 \)

Oil content (%) = \( \frac{w_1}{w} \times 100 \)

Oil load (%) = \( \frac{w_2}{w_3} \times 100 \)

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 crosslinker.

2.5.2. Oil release studies

A known quantity of microcapsules was placed into a known volume of 0.3% 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. An aliquot (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 256 nm (UV-2001 Hitachi) 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% Tween 80 solution was returned to the container.

Microcapsules were grounded and FTIR spectra were recorded using KBr pellet in a Nicholet (model Impact-410) spectrophotometer. Surface characteristics of the microcapsules were studied using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 15 kV.

3. Results and discussion

The minimum temperature and ratio of gelatin to sodium sulphate at which phase separation occurred were 40°C and 1:10 (data not shown). This was judged by the clear separation of gelatin in fine particle form from its aqueous solution. This temperature and ratio were maintained during preparation of microcapsules in the subsequent experiments. All experiments were carried out in triplicate and results presented were the average values.

3.1. Effect of variation of oil loading

The effect of variation of oil loading on the encapsulation efficiency and release rate is shown in Table I and Fig. 1. With the increase in oil loading, the release rate increased throughout the range of oil concentration studied. The encapsulation efficiency decreased while the % oil content and release rate increased. A possible explanation for the lower encapsulation efficiency at higher oil load might be due to the higher percentage of oil loss during isolation. At low oil load, the disperse force by the stirrer was more effective, causing the formation of smaller oil vesicles. The amount of gelatin present in the system was
Table 1
Effect of variation of oil loading, gelatin and glutaraldehyde concentration on the behaviour of microcapsules

<table>
<thead>
<tr>
<th>Sample particulars</th>
<th>Oil load (%)</th>
<th>Oil content (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin 5 mmol</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Glutaraldehyde 10 mmol</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Oil 10 mmol</td>
<td>30</td>
<td>30</td>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Gelatin: (2-5 gm); glutaraldehyde: (1-10 mmol/gm of gelatin); oil: (5-15 ml); water: 50 ml; temperature: 30 °C.

3.2. Effect of variation of gelatin concentration

Table 1 shows the effect of variation of gelatin concentration. As expected, oil loading (%) and oil content (%) decreased as polymer content increased. Encapsulation efficiency (%) increased first and then leveled off. With the increase in polymer content, more and more gelatin would be available to encapsulate the oil vesicles and thereby efficiency would be increased. At certain polymer content, all the oil vesicles present would be encapsulated by the polymer. After that, excess polymer would be used to thicken the microcapsule wall, which resulted in leveling off the efficiency. Fig. 2 shows the release profile with the variation of gelatin concentration. The concentration of gelatin was varied from 2 to 5 g. The release rate decreased with increase in gelatin concentration. The increase in wall thickness of the microcapsule might be responsible for this type of behavior.
3.3. Effect of variation of glutaraldehyde concentration

The related results are shown in Table 1 and Fig. 3. The trend of oil loading (%) and oil content (%) shown in the table was as per expectation. The increased encapsulation efficiency (%) could be due to the improvement in oil retention capacity of the microcapsules caused by the reaction between gelatin and glutaraldehyde. An increase in the degree of crosslinking, as expressed by molar concentration of glutaraldehyde used, resulted in a significant decrease in oil release rate throughout the glutaraldehyde concentration studied (1 mmol/g of gelatin -5 m mol/g of gelatin). As degree of crosslinking of gelatin increased, the microcapsule wall became denser resulting in the decrease of diffusion rate of oil through the microcapsule wall. Similar types of observations were reported in the literature (Bachtzi and Kipparissides, 1996; Raymond et al., 1990). Dinarvand et al. (2005) also investigated and reported the effect of crosslinker on the release rate of lactic acid from gelatin microspheres.

3.4. Scanning electron microscopic study

SEM photographs of glutaraldehyde crosslinked gelatin microcapsules of varying oil content are shown (Fig. 4). Microcapsules appeared to be made of spherical units linked to each other. The external surface appeared smooth at low oil loading indicating the formation of a continuous film by gelatin. At higher oil loading, a bursting look was observed. Chan et al. (2000) reported similar type of result while encapsulating two different type of oils in sodium alginate crosslinked matrix. The microcapsules prepared at
low oil loading, appeared dry and powdery on physical verification, whereas those prepared at higher oil loading appeared oily and agglomerated.

3.5 FTIR study

FTIR spectra of ZLO, gelatin, physical mixture of ZLO, glutaraldehyde, gelatin and ZLO containing crosslinked gelatin microcapsules were recorded (spectra not shown). Physical mixture was prepared using the ratio of ZLO, glutaraldehyde and gelatin similar to those of ratio used in preparing ZLO containing crosslinked gelatin microcapsules. In the spectra, the carbonyl stretching band of ZLO between 1637–1720 cm\(^{-1}\) remained almost unchanged in the case of physical mixture as well as microcapsule. The other notable peaks appeared at 1457 70 cm\(^{-1}\), 1377 78 cm\(^{-1}\), 1232 73 cm\(^{-1}\), 1167 53 cm\(^{-1}\) and 1019 89 cm\(^{-1}\), which were due to CH\(_2\) asymmetric deformation, CH\(_2\) symmetric deformation, C–N, C–C and C–O stretching vibration also remained almost unchanged in the physical mixture and microcapsules. These results indicated the absence of any significant interaction between the ZLO and the gelatin polymer.

4. Conclusion

It was concluded that oil from \textit{Z. limonella} could be encapsulated successfully within crosslinked gelatin microcapsule. The release rate was dependent on oil content, crosslinking density and encapsulating polymer concentration.

Acknowledgement

The authors thank Defence Research Laboratory, Tezpur for financial assistance.

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