Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex

Nirmala Devi, Dilip Kumar Kakati

Department of Chemistry, Gauhati University, Guwahati 781 014, India

A R T I C L E   I N F O

Article history:
Received 23 November 2012
Received in revised form 7 February 2013
Accepted 10 February 2013
Available online 26 February 2013

Keywords:
Biopolymers
Crosslinking
Electron microscopy
Microparticles
Swelling

A B S T R A C T

Porous microparticles of different sizes were prepared by polyelectrolyte complexation of biopolymers gelatine A and sodium alginate for microencapsulation of food bioactives. The optimum pH and ratio between the polymers sodium alginate and gelatine for maximum complexation was found as 3.7 and 1:3.5 respectively. Effect of various factors like amount of surfactant, concentration of polymer and cross-linker on the formation, size and porous/nonporous nature of the microparticles were investigated. The particles' diameter on swelling at pH = 7.4 was twice that at pH = 1.2 indicating the pH responsiveness. These microparticles were used as carrier for ascorbic acid. The surface morphology and sizes of the microparticles were investigated by scanning electron microscope (SEM). Fourier transform infrared spectroscopy (FTIR) study indicated the formation of polyelectrolyte complex between gelatine and sodium alginate and successful encapsulation of ascorbic acid into the microparticles. The microparticles were further characterized by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) study.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Microencapsulation technology as a prominent multidisciplinary area has its wide impact in many fields including pharmaceutical, agriculture, biotechnology, cosmetic, food and flavour industry. Thereby, the dimensions cover the range from millimetres to submicrometer scale, and the functionality ranges from simple storage devices to intelligent systems responding reproducibly to the environment (Mahou and Wandrey, 2010). Microencapsulation allows sensitive ingredients to be physically enveloped in a protective matrix or wall material in order to protect the ingredients or core materials from adverse reactions, loss or against light, heat and prolonged contact with air (Duclairoir et al., 2002). Wall materials must retain and protect the encapsulated core material from loss and chemical damage during manufacture, storage, handling and must subsequently release them into the final product during its manufacture or consumption (Kim et al., 2006). This technology has been extensively adopted for the practical applications for encapsulation of drugs, fertilizers, pesticides, repellents, vitamins, enzymes, fragrances, food and flavour (Devi and Maji, 2009a, 2010; Heris et al., 2012; Vaida et al., 2010), etc. The use of microparticles for either in the form of microsphere or microcapsules for controlled release of food bioactives such as vitamins, probiotics, bioactive peptides and antioxidants is a prospective field of interest in food, and some attempts have been made to fabricate microparticles for such application (Lee et al., 2009). For instance, Prasertmanakit et al. prepared ethyl cellulose microcapsules for protecting and controlled release of folic acid in foods and supplements (Prasertmanakit et al., 2009). Microencapsulation of haemoglobin in chitosan coated alginate microspheres was carried out to control the release of haemoglobin (Silva et al., 2006). Gladin nanoparticles were prepared and reported as suitable α-Tocopherol or Vitamin E drug release carrier (Duclairoir et al., 2002).

Ascorbic acid is a water-soluble vitamin, which is regarded as a vitamin supplement to reinforce dietary intake of vitamin C. Vitamin C is one of the most important antioxidants that may reduce the risk of cancer using various mechanisms (Esposito et al., 2002; Jacobs et al., 2001). However, environmental factors, such as temperature, pH value, oxygen, metal ion, UV and X-ray affect the stability of ascorbic acid (Alishahi et al., 2011; Kirby et al., 1991; Liao and Seib, 1988; Uddin et al., 2001). Moreover, acids can cause problems in conjunction with other food ingredients, such as decrease in flavour, undesirable odours and premature changes in pH (Trindade and Grosso, 2000). Microencapsulation technique has recently been utilized to reduce these problems (Desai and Park, 2005; Desai et al., 2006).

One of the most promising approaches to create food-grade microparticles is to use biopolymers, such as proteins and polysaccharides, as building blocks. Gelatin is biocompatible, biodegradable, edible and soluble at the body temperature and therefore it is an ideal material for food and pharmaceutical applications (Rokhade...
et al., 2006). It is positively charged below its isoelectric point and is expected to form polyelectrolyte complex with sodium alginate, which will have negative charges at lower pH (Almeida and Almeida, 2004; Saravanan and Rao, 2010; Shinde and Nagarsenker, 2009).

Thus, gelatin–alginate complex microparticles were prepared by water-in-oil (w/o) emulsification crosslinking method.

The objective of this study was to prepare and evaluate the polyelectrolyte complex of gelatin-A and sodium alginate as new microencapsulating material using genipin as crosslinker and ascorbic acid as model active agent. Genipin is a natural crosslinker which is less cytotoxic, biocompatible and feasible (Sung et al., 1999). Besides, to avoid the use of any toxic organic solvents as well as the most popularly used paraffin oil (Kumbar et al., 2002; Lucinda-Silva and Evangelista, 2003), this process involved water as a solvent and a vegetable oil (sunflower oil) as emulsion medium. Another purpose of this study was to investigate the optimal conditions for the formation of microparticles of desired size, porosity and hence the dependence of release rate of active agent on the reaction conditions.

2. Materials and methods

2.1. Chemicals

Gelatin type A (Sigma–Aldrich Inc., USA), sodium alginate (HiMedia Laboratories, India), ascorbic acid (HiMedia Solventary, India), glacial acetic acid (E. Merck, India), tween 80 (E. Merck, India) and genipin (Mol. wt. 226.22) (Challenge Bioproducts Co. Ltd., Taiwan) were purchased and used as received. Edible grade refined sunflower oil was purchased from local market. Double distilled water was used throughout the study. Other reagents used were of analytical grade.

2.2. Polyelectrolyte complexation, microparticle preparation and microencapsulation

Polyelectrolyte complexes may result on mixing oppositely charged polyelectrolyte in aqueous solution. The electrostatic interaction is a driving force to form a complex between the polycation and polyanion. The polyelectrolyte complex formation is dependent on many factors, among others molecular weights, polymer concentration, ratio of the two interacting polyelectrolytes, ionic strength and pH of the solution, and temperature. The optimal conditions (sodium alginate:gelatin = 1.0:3.5 and pH = 3.75) for the formation of polyelectrolyte complex of gelatin/sodium alginate were evaluated by determining the turbidity and relative viscosity at various ratio of sodium alginate to gelatin and at various pH.

In order to optimize the ratio of gelatin (type-A) and sodium alginate, the measurements of turbidity and viscosity are essential. The mixing of gelatin and sodium alginate in different ratios would produce solutions of different turbidity. The optimal ratio at which complete phase separation occurred between gelatin and sodium alginate was the point where the solution would have the maximum turbidity. The change in absorbance due to turbidity was monitored at a particular wavelength employing UV spectrophotometer (Model UV-2001, Hitachi). The viscosity of the supernatant solution was measured by using an ubbelohde viscometer at 30 °C. Polymer in the supernatant solution would be either negligible or absent when the interaction between sodium alginate and gelatin would be maximum. At this stage, the viscosity of the supernatant would be close or similar to the solvent viscosity. All the successive experiments were performed at this optimal pH and polymer ratio. The optimum ratio of sodium alginate to gelatin and pH at which maximum complexation occurred were 1.0: 3.5 and 3.75 respectively.

Polyelectrolyte complexation for microparticle preparation was carried out by a water-in-oil (w/o) emulsification and crosslinking method in a beaker fitted with a mechanical stirrer (Eltek, Electrotech, India). To the beaker containing a known amount of (180 ml) sunflower oil, 15 ml of gelatin-A solution (0.437–7.0%w/v) was added, under stirring condition (200–1800 rpm) at 60 ± 1 °C to form an emulsion. Tween 80 (0–1.0 g) was dissolved in water (10 ml) separately and was added to the beaker to stabilize the emulsion. A known amount of (15 ml) sodium alginate solution of concentration 0.125–2.0% (w/v) was added to the beaker drop wise to attain complete phase separation. However, the weight ratio of sodium alginate to gelatin was maintained at 1.0:3.5 during all the experiments. The pH of the mixture was then brought down to 3.7 by adding 5% (v/v) of the glacial acetic acid solution. The beaker containing the microparticles was left to rest at this temperature for approximately 15 min. The system was then cooled to 5–10 °C to harden the microparticles. The cross linking of the polymer microparticles was achieved by slow addition of certain amount of genipin (0.5225%) solution. The temperature of the beaker was then raised to 45 °C and stirring was continued for another 3 h to complete the crosslinking reaction. The microparticles were filtered through 300-mesh nylon cloth and washed with water. The microparticles were further washed with acetone to remove oil, if any, adhered to the surface of microparticles and freeze-dried.

The dried microparticles were then dipped in ascorbic acid (0.5–10%, w/v) in phosphate buffer of pH 7.4 for different times (0.5–24 h) in a nitrogen environment and in absence of light, filtered through 300-mesh nylon cloth, and quickly washed with water to remove the surface adhered ascorbic acid. The ascorbic acid-encapsulated microparticles were again freeze-dried and stored in a glass bottle in refrigerator. The flow chart for the fabrication of microparticles is illustrated in Fig. 1.

2.3. Calibration curve of ascorbic acid

A calibration curve is required for the determination of encapsulation efficiency and release rates of the microparticles. A known concentration of ascorbic acid in double distilled water was scanned in the range of 200–500 nm by using UV visible spectrophotometer. For ascorbic acid having concentration in the range 0.0001–0.001 g/100 ml, a prominent peak at 265 nm was noticed. The absorbance values at 265 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of ascorbic acid was obtained by knowing the absorbance value.

2.4. Loading efficiency

A known amount of accurately weighed ascorbic acid loaded microparticles were grounded in a mortar, transferred with precaution to a volumetric flask containing 100 ml of water (having pH = 7.4, maintained by phosphate buffer solution) and kept for overnight with continuous stirring to dissolve the ascorbic acid in the microparticles. The solution was collected and the ascorbic acid inside the microparticles was determined employing UV spectrophotometer. The loading efficiency (%) was calculated by using the calibration curve and the following formulae (Devi and Maji, 2009b).

\[
\text{Loading efficiency} \ (\%) = \frac{w_1}{w_2} \times 100
\]

where \( w_1 \) = amount of ascorbic acid encapsulated in a known amount of microparticles, and \( w_2 \) = weight of microparticles.

2.5. Ascorbic acid release studies

Ascorbic acid release studies from the ascorbic acid encapsulated microparticles were carried out by using UV–visible spectrophotometer (Model UV-2001, Hitachi). A known quantity of ascorbic acid...
acid loaded microparticles was taken into a known volume (100 ml) of water having different pH (pH = 1.2 and 7.4). This pH was maintained by using HCl and phosphate buffer solution. The content was shaken from time to time and the temperature maintained throughout was 30 °C (room temperature). An aliquot sample of known volume (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 265 nm for the determination of cumulative amount of ascorbic acid release up to a time t. The experiments were performed in triplicate. To maintain a constant volume, 5 ml of the solution having same pH was returned to the container.

2.6. Water uptake study

The swelling behaviour of sodium alginate–gelatin microparticles were carried out at pH 1.2, 6.6 and 7.4 using 0.1N HCl and phosphate buffer. The pre-weighed microparticles were immersed in either 0.1N HCl (pH 1.2) or phosphate buffer at different pH (pH 6.6 and 7.4). The swollen microparticles were weighed for 48 h. Every time, the weighing of the swollen microparticles was carried out after their surfaces were dried with filter paper to remove droplets on the surface. The swelling behaviour was determined by measuring the change of the weight of the microparticles. Swelling index for each sample determined at time t was calculated using the following equation (Khan et al., 2010).

\[
\text{Swelling index (\%) = } \left( \frac{W_s - W_0}{W_0} \right) \times 100
\]

where \( W_s \) is the weight of the swollen microparticles after allowing to swell for a time (t) and \( W_0 \) initial weight of the microparticles before swelling. The experiments were performed in triplicate and the swelling index values reported here are the average ones.

2.7. Scanning electron microscopy study

The samples were deposited on a brass holder and sputtered with platinum. The detailed morphology and sizes of the microparticles were studied at room temperature using scanning electron microscope (Model JSM-6360, JEOL) at an accelerated voltage of 20 kV.

2.8. Optical microscopy study

The morphology and swelling behaviour of microparticles were observed using optical microscope (Model Carl Zeiss Micromaging, GmbH). Dry and swelled microparticles were placed onto a glass slide, observed under microscope and captured.

2.9. Fourier transform infrared (FTIR) study

FTIR spectra were recorded using KBr pellet in a FTIR spectrophotometer (Model Impact-410, Nicholet). Gelatin A, sodium alginate, polyelectrolyte complex of gelatin A and sodium alginate, ascorbic acid and ascorbic acid loaded microparticles were each separately finely grounded with KBr and FTIR spectra were recorded in the range of 4000–400 cm\(^{-1}\).

2.10. X-ray diffraction study

X-ray powder diffractograms of sodium alginate, gelatin A, microparticles, ascorbic acid and ascorbic acid encapsulated microparticles were recorded on an X-ray diffractometer (Model Mini-flex, Rigaku). The samples were scanned between 2θ = 3° and 60° at the scan rate of 4°/min.

2.11. Thermal property study

Thermal properties of the samples were studied by employing thermogravimetric analyser (TGA) and differential scanning calorimeter (DSC). TGA thermograms of sodium alginate, gelatin, gelatin–sodium alginate microparticles, ascorbic acid and ascorbic acid loaded microparticles were recorded by employing a thermogravimetric analyzer (Model TGA-50, Shimadzu) in the temperature range of 30–600 °C at a heating rate of 10 °C/min in a nitrogen atmosphere. DSC study was carried out in a differential scanning calorimeter (Model DSC-60, Shimadzu) at a heating rate of 10 °C/min up to 300 °C. The investigations were done under nitrogen atmosphere.

3. Results and discussion

3.1. Behaviour of the system as a function of pH, polymer ratio and temperature

The interaction between gelatin A and sodium alginate is expected to be electrostatic in nature. Indeed, because the pH influences the ionization of the protein charges, electrostatic complexes would be formed in the pH window where gelatine and sodium alginate are oppositely charged. Furthermore, ratio between the two biopolymers is obviously of importance in electrostatic complex formation. At a certain pH, there exists a specific ratio of gelatin and sodium alginate, at which electroneutrality of the complex would be formed in the pH window where gelatine and sodium alginate are oppositely charged. The detailed morphology and sizes of the microparticles were studied at room temperature using scanning electron microscope (Model JSM-6360, JEOL) at an accelerated voltage of 20 kV.

The morphology and swelling behaviour of microparticles were observed using optical microscope (Model Carl Zeiss Micromaging, GmbH). Dry and swelled microparticles were placed onto a glass slide, observed under microscope and captured.

The interaction between gelatin A and sodium alginate is expected to be electrostatic in nature. Indeed, because the pH influences the ionization of the protein charges, electrostatic complexes would be formed in the pH window where gelatine and sodium alginate are oppositely charged. Furthermore, ratio between the two biopolymers is obviously of importance in electrostatic complex formation. At a certain pH, there exists a specific ratio of gelatin and sodium alginate, at which electroneutrality of the complex would be formed in the pH window where gelatine and sodium alginate are oppositely charged. The detailed morphology and sizes of the microparticles were studied at room temperature using scanning electron microscope (Model JSM-6360, JEOL) at an accelerated voltage of 20 kV.

The morphology and swelling behaviour of microparticles were observed using optical microscope (Model Carl Zeiss Micromaging, GmbH). Dry and swelled microparticles were placed onto a glass slide, observed under microscope and captured.

The interaction between gelatin A and sodium alginate is expected to be electrostatic in nature. Indeed, because the pH influences the ionization of the protein charges, electrostatic complexes would be formed in the pH window where gelatine and sodium alginate are oppositely charged. Furthermore, ratio between the two biopolymers is obviously of importance in electrostatic complex formation. At a certain pH, there exists a specific ratio of gelatin and sodium alginate, at which electroneutrality of the complex would be formed in the pH window where gelatine and sodium alginate are oppositely charged. The detailed morphology and sizes of the microparticles were studied at room temperature using scanning electron microscope (Model JSM-6360, JEOL) at an accelerated voltage of 20 kV.

The morphology and swelling behaviour of microparticles were observed using optical microscope (Model Carl Zeiss Micromaging, GmbH). Dry and swelled microparticles were placed onto a glass slide, observed under microscope and captured.
solution slowly to sodium alginate solution to make the total volume of 45 ml. The mixtures were incubated at 40 °C for 24 h. The formation of complexes was monitored by turbidity measurement as a function of the different polymer ratios. The supernatant solution was separated and viscosity of the supernatant was measured. Each measurement was done in triplicate and the results reported were the average values.

Fig. 2a shows the change in supernatant viscosity with variation in percentage of gelatin in gelatin–alginate mixture. Viscosity was found to decrease initially, reaching a minimum value, and after that it increased slightly with the increase in the percentage of gelatin. The minimum viscosity observed when the percentage of gelatin in the mixture was 77.77%. At this percentage of gelatin, both the polymers probably reacted maximum to form polyelectrolyte 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 gelatin in the supernatant.

Fig. 2b shows the change in supernatant absorbance with variation in percentage of gelatin in gelatin–alginate mixture. Absorbance was found to decrease initially, reaching a minimum value, and after that it increased slightly with the increase in the percentage of gelatin. The minimum absorbance observed when the percentage of gelatin in the mixture was 77.77%. At this percentage of gelatin, both the polymers probably reacted maximum to form polyelectrolyte 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 gelatin in the supernatant.

Similarly, for optimization of pH, solution of gelatin (0.5% w/v) and sodium alginate (0.5% w/v) were prepared in water and both the solutions were mixed in a definite ratio (gelatin: alginate = 3.5:1). The pH of the mixing solution was varied from 2.0–5.0 by using 5.0% glacial acetic acid. The effect of variation of pH on relative viscosity and turbidity of the supernatant was depicted in Fig. 3. The minimum relative viscosity (Fig. 3a) and minimum turbidity (Fig. 3b) of the supernatant were found to appear at pH 3.70. This implied that the complexation between the two polymers was highest at this pH. The explanation for this was similar to that of given earlier. All the subsequent microencapsulation reactions were carried out at this pH and ratio between the polymers.

Temperature change, in general influences the biopolymer/biopolymer interactions by changing the Flory–Huggins interaction energy. If other enthalpic interactions—in addition to the Coulombic interactions—would be involved, the temperature would also have an influence (Weinbreck et al., 2004). In order to get clear Gelatin A solution, it should be heated slightly to the temperature of 40 °C. If the gelatine solution was kept for overnight at room temperature (28 °C), it turned to gel and it should be heated again to get solution from the gel. Therefore, in this study, effect of variation of temperature on polyelectrolyte complexation was carried out in the temperature range 40–60 °C. The polymer solutions were mixed at definite ratio between the polymers (gelatin: alginate = 3.5:1) and pH was maintained at 3.70. Keeping these two parameters constant temperature was varied from 40–60 °C for different samples and turbidity was monitored. In the range studied, temperature had practically no effect on the turbidity and hence on insoluble complex formation. Thus the polyelectrolyte complexation in this study resulted mainly due to Coulombic interactions.

3.2. Effect of variation of crosslinker concentration and stirring

The formation of gelatine–alginate microparticles in sunflower oil was significantly dependent on the crosslinker and mechanical...
stirring (Devi and Maji, 2009b). SEM micrographs of microparticles prepared by using different amount of crosslinker, genipin were shown in Fig. 4. Without the addition of crosslinker, no microparticles were formed. Instead, the whole polymer became a lump like product (Fig. 4a). Thus crosslinking was necessary to harden the soft aqueous phase microparticles formed within the oil phase. Without proper crosslinking there was always chance of aggregation of the soft microparticles to produce polymer lump. With the increasing of the amount of crosslinker, the microparticles progressively developed proper shape from aggregated (Fig. 4b) to relatively less aggregated (Fig. 4c) and to free-flowing spherical microparticles (Fig. 4d). Similarly, stirring speed also affected the nature. At low stirrer speed (200 rpm), the agglomeration of particles was more compared to those of particles produced at higher stirrer speed (1800 rpm) (figure not shown). Improper mixing of polymers at low stirrer speed might be responsible for the observed agglomeration. Zhuo et al. reported similar observations during studying the particle size of polyurea microcapsules by interfacial polymerization of polyisocyanates (Zhuo et al., 2004).

3.3. Effect of variation of amount of surfactant

The effect of surfactant on the morphology of the prepared gelatine–alginate microparticles was profound in two ways– the microparticle size and the porosity of the microparticles. The formation and particle size of each microparticle depends on the size of the dispersed droplet, which is determined by the surfactant (tween 80) used and the emulsifying conditions. Surfactant tween-80 had important role in stabilizing the microparticles of gelatine–alginate complex formed in sunflower oil. A matrix gel like product was formed if surfactant was not added. But different sizes of microparticles were formed on addition of varying amount of surfactant. SEM photographs of the microparticles were shown in Fig. 5. With the increase of amount of tween-80 from 0.037 g to 0.740 g/g of polymer, the sizes of the microparticles decreased as shown in Fig. 5a–f. At higher concentration of surfactant, the aqueous phase is easily dispersed into finer droplets, owing to the higher activity of the surfactant, which would result in a lower free energy of the system, and lead to a smaller particle size (Devi and Maji, 2009b).

Moreover, it is well known that when the concentration of the surfactant increases to critical micelle concentration, surfactant molecules will get together and form micelles. When the aqueous phase containing micelles was dispersed in the continuous oil phase to form small water droplets, the micelles would absorb oil from the oil phase. Therefore, when the aqueous phase of polymer formed microparticles, some oil was also remained absorbed. In the subsequent crosslinking and hardening some of the absorbed oil would leak out and form porous microparticles. This porosity was enhanced further in washing step of the microparticles with acetone to remove surface adhered oil. This was the reason behind the increasing porosity of the microparticles on increasing the amount of surfactant (Fig. 5a–f). The inset pictures (Fig. 5ai and ei) were the enlarged SEM micrographs of surface of the microparticles (Fig. 5a and e respectively). Microparticles prepared with less amount of tween 80 possessed nonporous surface (Fig. 5ai) whereas microparticles with relatively higher amount of tween 80 had porous surface (Fig. 5ei). Similar types of observations were reported by Zhou et al. (2007).

3.4. Effect of variation of polymer concentration

The effect of variation of polymer concentration on the surface morphology and size of the microparticles was shown in Fig. 6.
With decrease of amount of polymer from 1.35 g to 0.168 g, a decrease in the size of the microparticles was observed (Fig. 6a–d). In the presence of higher amount of polymer, the surfactant present might not be capable of covering all the surfaces of the microparticles properly. This resulted in the coalescence of some of the microparticles and led to the formation of larger microparticles. Besides this, the dispersive force of the stirrer became less efficient in presence of higher amount of polymer and as a result larger microparticles formed (Fig. 6a–d).

On decreasing the amount of polymer or increasing the dilution of the polymer solution, the porosity of the microparticles increased (Fig. 6a–d) and this might be due to the evaporation of water from the thin polymer surface of microparticles formed from dilute polymer solution. Further, it could be expected that the highly concentrated polymer solution increased not only the initial viscosity of the polymer phase, but also the rate of polymer solidification. Apparently, rapid solvent removal resulted in a homogenous viscous polymer layer at the microparticle droplet interface. As a consequence, the surface layer of polymer was solidified in a nonporous morphology (Crotts and Park, 1995) (Fig. 6a) for increased concentration of polymer. On further dilution of polymer solution from 0.168 g (Fig. 6d) to 0.084 g, breaking of particles occurred (Fig. 6f) which might be due to the excess increase of pore size/or porosity and insufficient amount of polymer to form microparticles. However, 0.168 g of polymer could produce relatively smaller and less porous microparticles on decreasing the amount of tween 80 (Fig. 6e).

3.5. Effect of washing and drying

Washing step and type of drying had strong impact on the surface morphology of the microparticles as shown in Fig. 7. Indeed, during the washing of microparticles with water and acetone, the surface adhered and some of the trapped oil droplets, the unreacted crosslinker and some of the uncomplexed polymers, if any are extracted from the particles, giving rise to the porous structure of the microparticles (Fig. 7a). Thus, the trapped oil domains in the surface behave as true porogens leading to the formation of porous structure. Peniche et al. reported similar type of observations while studying the porous microspheres of acrylic acid in the presence of chitosan (Peniche et al., 2003). These were freeze-dried particles. The porous particles if, on the other hand, dried under low vacuum;
they tend to break (Fig. 7b). More prominent effect of drying under vacuum was observed when microparticles of both medium polymer concentration (Fig. 7c) and high polymer concentration (Fig. 7d) were dried under high vacuum as both of these microparticles were broken severely.

3.6. Water uptake study

Fig. 8 showed the water uptake of neat gelatin–alginate polyelectrolyte complex matrix without crosslinking as a function of time of immersion in water in different pH media simulating gastrointestinal (GIT) pH of 1.2, 6.6 and 7.4. The experiment was followed with the polymer complex matrix sheet in order to improve reproducibility. The swelling was rapid up to 1 h in all the acidic and basic pH. However, matrices showed more swelling at around neutral medium (pH 6.6 and 7.4) and less swelling in acidic medium (pH 1.2). The swelling indices of the uncrosslinked matrix at different pH's followed the pattern as 1.2 < 6.6 < 7.4. The swelling pattern of crosslinked microparticle samples at pH 1.2 and 7.4 was shown in Fig. 9. Initially the swelling was high for 5 h, then slowly increased till 48 h and finally almost leveled off. Microspheres with higher crosslinking showed lesser water uptake than the microparticles with low crosslinking. This was due to the formation of more compact wall (Agnihotri and Aminabhavi, 2004) caused by crosslinking. Yet again, water uptake was more at pH 7.4 compared to at pH 1.2 as found earlier while studying in swelling behaviour of uncrosslinked complex. The swelling index almost constant after 24 h in the time duration of study without any breakage of the sample till 96 h. This behaviour is related to the ionisable character of the amine groups of gelatine which are positively charged at lower pH due to which they can complex with alginate which is negatively charged. Microparticles formed by the polyelectrolyte complexation between gelatin A and sodium alginate became more stable probably at lower pH. At higher pH, the tendency to decomplexation between gelatin and sodium alginate might be responsible for the higher water uptake and consequent breakage of the complex. Similar findings were reported by Liu et al. (Liu et al., 2004) during studying the swelling behaviour of gelatin-DNA semi-interpenetrating polymer network at different pH.
at pH 7.4 was almost twice than that at pH 1.2. That was also confirmed by allowing some randomly chosen microparticle to swell at pH 1.2 and 7.4 and studied under optical microscope (Fig. 10). The microparticle’s diameter after 6 h of swelling at pH 7.4 was more than twice that at pH 1.2 for low crosslinked particle (Fig. 10a–c) but for relatively high crosslinked the particle diameter was almost twice under same conditions of study. This behaviour makes the particles attractive in applications where inhibition of the release in gastric environment (low pH values) is desired. Besides, the pH responsive behaviour of the particles might be suitable for smart drug delivery.

3.7. Effect of variation of crosslinker and ascorbic acid concentration on loading efficiency

The effect of variation of concentration of ascorbic acid and immersion time of microparticles on loading efficiency was studied. For all these experiments, the microparticles prepared by using same amount of surfactant and polymer were taken. Some initial experiment revealed that loading efficiency increased with the increase in immersion time till 5 h but above that immersion time had no distinct effect on loading efficiency. Moreover, for fixed immersion time, the loading efficiency was found to increase.
with the increase in the concentration of ascorbic acid and this was due to the more diffusion of ascorbic acid into the microparticles. Again, higher the amount of crosslinker in the microparticles, the lower was the loading efficiency. The decrease in loading efficiency might be attributed to the formation of more compact wall due to crosslinking that led to decrease in diffusion rate. Therefore, all the crosslinked samples with different amount of crosslinker in range of study were immersed for 24 h and 10% ascorbic acid solution in phosphate buffer at pH 7.4 to attain equilibrium swelling. At this pH, the microparticles absorbed and entrapped ascorbic acid while swelling. These microparticles after freeze drying showed loadings around 18–23% in weight as determined by UV spectroscopy.

3.8. Effect of variation of cross-linker on release rate of ascorbic acid

The effect of variation of crosslinker concentration (0.8–2.5 mmol/g of polymer) on release rate at pH 1.2 and 7.4 is shown in Fig. 11. Microparticles having approximately similar loading were chosen for the study of the release rate at different pH. The release rate of ascorbic acid was found to decrease with the increase in the amount of crosslinker in the microparticles. There was a moderated burst effect initially in all the cases, reached maximum and leveled off finally. The compact microparticle wall was responsible for the decrease in release rate as explained earlier. Moreover, the release at pH = 1.2 was less compared to that of at pH = 7.4. The different swelling behaviour of the microparticles in different pH medium was responsible for this kind of release rate.

3.9. Fourier transform infrared (FTIR) study

The FTIR analysis of the prepared microparticles is shown in Fig. 12 together with the spectra of gelatin A and sodium alginate used as references. Gelatin has positive charge at acidic pH due to presence of amino groups. Sodium alginate has free carboxyl group that imparts negative charge to these molecules. During polyelec-
trolyte complexation carboxyl groups in polysaccharides interact with amino groups in protein to form a complex that contains amide (Saravanan and Rao, 2010). Formation of amide due to interaction of free carboxyl and amino groups present in alginate and gelatin respectively can be studied using FTIR spectra. FTIR spectrum of gelatin (Fig. 12a) revealed the presence of characteristic functional group at 3443 cm\(^{-1}\) for amino group. In the spectrum of gelatin the other notable peaks observed were at 2917 cm\(^{-1}\) (C–H stretching of alkenes), 2823 cm\(^{-1}\) (C–H stretching of alkanes) and 1612 cm\(^{-1}\) (amide-I, CO and CN stretching). Other peaks observed were at 1152 cm\(^{-1}\), 1030 cm\(^{-1}\) due to, C–O stretching of carboxylic acid and C–N stretching of amines respectively. In the spectrum of sodium alginate (Fig. 12b) the following peaks were observed at 3452 cm\(^{-1}\), 1603 cm\(^{-1}\), 1096 cm\(^{-1}\), 1021 cm\(^{-1}\), which were assigned due to alcoholic O–H stretching, carboxylate salt asymmetric stretch and C–O stretching of ether respectively. The peaks of free amino groups that present in gelatin broadened and showed decreased intensity in gelatin–alginate complex microparticles (Fig. 12c). A characteristic peak for amide in the region of 1500–1650 cm\(^{-1}\) appeared in the complex (1593 cm\(^{-1}\) due to C–O stretching for amide) and confirmed formation of complex due to reaction between amino group of gelatin and carboxylic group of alginate. Moreover, the shift of the peak of amide I from 1612 cm\(^{-1}\) to 1593 cm\(^{-1}\) observed in the complex microparticles of gelatin and sodium alginate also indicated that the negatively groups of alginate might associate with positively charged gelatin. Furthermore, clearly explaining, the carbonyl area split into three clear peaks at 1744 cm\(^{-1}\), 1593 cm\(^{-1}\), 1501 cm\(^{-1}\), which could be assigned to the C=O stretching vibration, and to the NH\(_3\) and COO\(^{-}\) groups characteristics of the interpolyelectrolyte complex as it was broadly described in the literature (Muyona et al., 2004; Peniche et al., 2003; Pranoto et al., 2007). The FTIR spectrum of ascorbic acid (Fig. 12d) showed the following characteristic peaks at 3525 cm\(^{-1}\), 3414 cm\(^{-1}\), 1755 cm\(^{-1}\), 1681 cm\(^{-1}\), 1319 cm\(^{-1}\), 1149 cm\(^{-1}\), 1121 cm\(^{-1}\) and 1026 cm\(^{-1}\). The peaks at 1149 cm\(^{-1}\), 1319 cm\(^{-1}\) and 1681 cm\(^{-1}\) were assigned due to stretching and bending vibration of C=O and OH groups present on ascorbic acid. The FTIR spectrum of the ascorbic acid loaded microparticles (Fig. 12e) showed these characteristic peaks (shown by arrows) but comparatively broader peaks at 3425 cm\(^{-1}\), 1755 cm\(^{-1}\), 1651 cm\(^{-1}\), 1149 cm\(^{-1}\), 1121 cm\(^{-1}\), 1026 cm\(^{-1}\), which resulted in the appearance of super-imposed peaks of ascorbic acid and gelatine–alginate microparticles at these regions. This confirmed successful encapsulation of ascorbic acid with retained stability after encapsulation as well as absence of any significant interaction between ascorbic acid and polymer.

3.10. Thermal property study

TGA thermograms of sodium alginate (curve-a), gelatin A (curve-b), microparticles of polyelectrolyte complex of both the polymers (curve-c), ascorbic acid (curve-d) and microparticles loaded with ascorbic acid (curve-e) are shown in Fig. 13. The decomposition temperature of neat sodium alginate and gelatin A was lower than that of crosslinked microparticles. However, sodium alginate showed lower thermal stability than gelatin A. Both sodium alginate and gelatin A showed similar type of weight loss pattern before decomposition but sodium alginate showed lower weight loss than gelatin after decomposition. Ascorbic acid decomposed after melt showing sharp decomposition and higher as well as almost complete weight loss. But it showed no weight loss before decomposition. The TGA thermograms of both the crosslinked microparticles and ascorbic acid loaded crosslinked microparticles showed similar type of decomposition and weight loss pattern. However, both showed higher thermal stability than the neat gelatin A and sodium alginate. The higher thermal stability of crosslinked microparticles might be due to the lower chance of elimination of small molecules like CO\(_2\) and CO with the formation of crosslinking, which acted as an insubble support and provided thermal resistance to the microparticles.

DSC thermograms of crosslinked sodium alginate–gelatin complex microparticles (curve-a), ascorbic acid (curve-b) and ascorbic acid loaded microparticles (curve-c) are shown in Fig. 14. The thermogram of ascorbic acid showed an endothermic peak at around 152 °C due to melting. There was no characteristic peak of ascorbic acid in the thermogram of ascorbic acid loaded microparticles. These results indicated that ascorbic acid was dispersed in the microparticles. Similar observation was reported by Patil and Sawant during DSC analysis of carvedilol drug encapsulated within alginate microspheres (Patil and Sawant, 2009).

3.11. X-ray diffraction (XRD) study

The XRD study facilitates to realize the crystalline or amorphous nature of core material in the polymeric matrix. The X-ray
diffractograms of sodium alginate (curve-a), gelatin A (curve-b), sodium alginate–gelatin microparticles (curve-c), ascorbic acid loaded microparticles (curve-d) and ascorbic acid (curve-e) are shown in Fig. 15. Sodium alginate, gelatin and microparticles showed no characteristics peaks implying amorphous nature of the polymers. Ascorbic acid exhibited multiple characteristic sharp peaks at 2θ varying from 5° to 60° which were due to the crystalline nature of ascorbic acid. Gelatin–sodium alginate microparticles did not exhibit any crystalline peaks. The characteristic crystalline peaks of ascorbic acid disappeared after encapsulation in crosslinked sodium alginate–gelatin microparticles but instead only microparticles pattern was obtained. This indicated that ascorbic acid was dispersed at the molecular level in the sodium alginate–gelatin microparticles.

4. Conclusion

Porous microparticles can be prepared by using the polyelectrolyte complex of the two biopolymers gelatine A and sodium alginate. The optimum conditions for maximum complexation between gelatine and sodium alginate were observed at sodium alginate to gelatin ratio of 1.0:3.5 by weight and pH 3.7. Microparticles of various sizes and porosity were prepared by varying surfactant and polymer concentration. The swelling of microparticles was pH dependent. Higher pH of the medium favoured the release of ascorbic acid in comparison to lower pH. FTIR study indicated complexation between gelatine and sodium alginate as well as the loading of ascorbic acid into the microparticles. TGA study revealed improvement of thermal stability due to crosslinking. DSC and XRD studies showed that ascorbic acid was dispersed at molecular level in the microparticles. The porous and pH responsive gelatine–sodium alginate microparticles are likely to have potential applications in food encapsulation, smart drug delivery, separation of biomolecules, enzyme immobilization, etc.

Acknowledgements

The research has been sponsored by the University Grants Commission, New Delhi, India under the UGC Dr. D.S. Kothari Post-Doctoral fellowship scheme. The author (N.D.) is grateful to UGC for financial assistance. The authors wish to thank SAIF, North-Eastern Hill University, Shillong, India for SEM analysis and Tezpur University, Napaam, India for TGA, DSC and XRD analysis.

References


