Study of Complex Coacervation of Gelatin A and Sodium Alginate for Microencapsulation of Olive Oil

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Complex coacervation of gelatin A and sodium alginate was carried out to obtain the maximum coacervate yield. Turbidity and coacervate yield (%) measurements were carried out to support the ratio of the two polymers and pH that produced maximum coacervation. The optimum ratio between gelatin A-sodium alginate and pH to form the maximum coacervate complex was found to be 3.5:1 and 3.5–3.8, respectively. Olive oil microencapsulation was carried out at the optimized ratio and pH. Microcapsules were crosslinked by using glutaraldehyde. Scanning electron microscopy studies confirmed the formation of free flowing spherical microcapsules of different sizes. The size of microcapsules increased with the increase in the concentration of the polymer. The encapsulation efficiency and the release rates of olive oil were dependent on the amount of crosslinker, oil loading and polymer concentration. Thermogravimetric study revealed improvement of thermal stability with crosslinking. Fourier Transform Infrared Spectroscopy study showed that there was no significant interaction between olive oil and gelatin-alginate complex.

Keywords: Complex coacervation, microcapsule, gelatin, sodium alginate, crosslinking

1 Introduction

The microencapsulation of active ingredients and their delivery holds promise for improved therapeutics. Polymer hydrogel submicrometer-sized capsules are finding widespread importance in the delivery of encapsulated toxic or fragile drugs (1). However, encapsulation of lipophilic drug is a problem in the case of water soluble natural polymers as the shell material. A liquid core or template composed of encapsulated oil serves as an ideal solvent for lipophilic drugs (2). Besides, olive oil contains essential vitamins, fatty acids and other natural nutrients (3). Olive oil as reported have rapid digestibility, anti-ulcer, anti-aging, stress and plasma cholesterol lowering properties as well as therapy potentials for diabetes and skin care (4–8). In addition to their known properties as nutraceuticals, cardioprotective agents and in skin care, new healthy properties are being extensively reported (9). In spite of all the proven potentiality, it has poor storage stability (10). Oxidative degradation results in a loss of nutritional quality and a development of undesired flavors. Therefore, oil encapsulation may be useful to retard lipid auto-oxidation (4) and increase the range of applications where otherwise oil could not be used (11). Several works has been devoted to address all these issues (12–14). Avoiding fatty acids profile alteration, through a microencapsulation process, will prevent losing such healthy properties of olive oil and increase self life during product manufacturing (11). Thus, microencapsulation of olive oil has the chance to enhance economic value, as health foods and may serve a therapeutic purpose.

Synthetic as well as natural polymers are used for microencapsulation and controlled release of active ingredients (15–17). But the recent trend has been to shift towards natural polymers due to their non-toxicity and biodegradability (18–19). Gelatin is the denatured collagen and has been widely used as a fundamental material for microspheres (20), sealants (21), tissue adhesives (22) and carriers for controlled delivery systems (23). Gelatin has also been widely used in combination with other polymers for encapsulation (24–25). Sodium alginate, sodium salt of alginic acid is white to yellowish-brown in colour, extracted from the cell of brown algae is widely used in various dosage forms and in the area of drug delivery systems (26). It is a linear copolymer with homopolymeric blocks of (1–4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks), or randomly organized blocks (26). It is suitable for oral consumption (27). It has been used as an encapsulating material for the
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microencapsulation of oils (28), drugs (28–29) and pesticides (30).

Complex coacervation can be induced in systems containing cationic and anionic hydrophilic colloids. Gelatin, an amphoteric protein, which is positively charged below its isoelectric point and is expected to form complex coacervate with sodium alginate, which will have negative charges at lower pH (31).

The objective of the present investigation is to study complex coacervation of gelatin A with sodium alginate and microencapsulation of olive oil in it. Efforts have also been made to study the release characteristics of oil from microcapsules prepared under different conditions.

2 Experimental

2.1 Materials

Gelatin type A, from porcine skin was purchased from Sigma Aldrich Inc. (USA). Sodium alginate and olive oil were purchased from Himedia Laboratories, Mumbai (India). Glacial acetic acid (E. Merck, India), Tween 80 (E. Merck, India), Glutaraldehyde 25% w/v (E. Merck, Germany) were used without further purification. Double Distilled ionized (DDI) water was used throughout the work. All other chemicals used were of analytical grade.

2.2 Microencapsulation Procedure

Encapsulation of olive oil was carried out under different reaction conditions by varying the content of polymers, oil, and crosslinker. Each polymer solution was prepared in distilled water. A known volume (140 ml) of 1–4% (w/v) of gelatin solution was taken in a beaker. This solution was stirred by mechanical stirrer under high agitation at 60 ± 1°C. This temperature was maintained throughout the experiment. Olive oil (0.5–7.0 g) was then added to this solution. After the addition of the oil a known volume (40 ml) of sodium alginate 1–4% (w/v) was added dropwise to the solution to attain complete phase separation. Thus the optimized weight ratio for gelatin and alginate (3:5:1) for coacervate formation was maintained for all the experiments. After the complete addition of sodium alginate, the beaker containing the solution was left at 60°C under stirring for almost 15 min. At this ratio, interaction between gelatin and sodium alginate took place completely as judged by the coacervate % yield and turbidity measurements. The pH of the mixture was then brought down to 3.75 by adding 2.5% (v/v) glacial acetic acid solution. At this pH, the yield was maximum as judged by % yield and turbidity measurements. The solution is then cooled to 5–10°C to harden the microcapsules. The microcapsules thus formed in the solution were crosslinked by slow addition of certain amount of glutaraldehyde (1.25–12.0 mmol). The temperature of the beaker was then raised to 45°C and stirring was continued for another 3–4 h to complete the crosslinking reaction. The solution was then cooled to room temperature slowly while stirring. The microcapsules were filtered out and washed with water. The microcapsules were further washed quickly with n-hexane in order to remove any oil adhered to the surface of the microcapsules. The microcapsules were freeze-dried and stored inside a refrigerator in a glass ampoule. The preparation process of olive oil loaded microcapsules is schematically presented in Figure 1.

2.3 Measurement of Turbidity and Coacervate Yield

The optimum ratio of gelatin A and sodium alginate for the formation of maximum yield of coacervate complex was determined by measuring the turbidity and % yield. The mixing of gelatin and alginate in different ratios would produce solutions of different turbidity. The optimal ratio at which complete phase separation occurred between gelatin and alginate was the point where the solution would have the maximum turbidity as well as maximum coacervate yield. Turbidity measurements were carried
Fig. 2. Effect of variation of gelatin concentration in gelatin–sodium alginate mixture on (a) turbidity of the supernatant solution (b) coacervate yield (%).

out in a Nephelo Turbidity Meter 131 (Systronics). The reference solution taken was a mixture of hexamethylenetetramine and hydrazine sulphide. The % coacervate yield was calculated using the following equation:

\[
\text{% yield} = \frac{\text{Experimental yield}}{\text{Theoretical yield}} \times 100
\]

The complex formation is highly pH dependent and so both the polymer solutions were prepared in buffer solutions. 0.5% (w/v) solution of each polymer, gelatin and sodium alginate, were prepared in 0.2 M sodium acetate buffer solutions. The coacervate yield (%) obtained by mixing of gelatin and alginate in different ratios was measured gravimetrically. The coacervates remained after decantation of supernatants was washed with distilled water and then dried at 40°C till the attainment of constant weight.

For optimization of pH, the ratio between the two polymers was fixed and pH was varied by adding solution of acetic acid (2.5% v/v) and sodium hydroxide (1% w/v).

2.4 Calibration Curve of Olive Oil

A calibration curve is required for the determination of release rate of olive oil from the microcapsules. It was found that n-hexane could easily dissolve a sufficient amount of olive oil. A known concentration of olive oil in hexane was scanned in the range of 200–800 nm by using a UV visible spectrophotometer. For olive oil having concentration in the range 0.001 to 0.1 g/100 ml, a prominent peak at 228 nm was noticed. The absorbance values at 228 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of olive oil was obtained by knowing the absorbance value.

2.5 Encapsulation Efficiency

The encapsulated microcapsules were treated with hexane (25 ml) and were stirred with magnetic stirrer for about 10 h. After the extraction of the olive oil the microcapsules were separated and the hexane solution containing the olive oil was kept in an oven, at a boiling temperature of hexane (69°C), in order to remove the hexane. The oil encapsulated was calculated gravimetrically and as well as by using the calibration curve. The encapsulation efficiency (%), oil content (%) and oil loading (%) were calculated by and the following formulae (32–34):

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

\[
\text{Oil content (%) } = \left(\frac{w_1}{w}\right) \times 100
\]

\[
\text{Oil load (%) } = \left(\frac{w_2}{w_3}\right) \times 100
\]

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

2.6 Oil Release Studies

Oil release studies of encapsulated oil were done by using UV–visible spectrophotometer (UV-1800 Shimadzu). A known quantity of microcapsules was placed into a known volume of hexane. The microcapsule-hexane mixture was shaken from time to time and the temperature throughout was maintained at 30°C (room temperature). An aliquot sample of known volume (5 ml) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 228 nm for the determination of cumulative amount of oil release up to a time t. Each determination was carried...
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Fig. 4. Scanning electron micrographs of (a) neat gelatin–sodium alginate complex (b) microcapsules without glutaraldehyde; microcapsules loaded with (c) oil = 2 g, polymer = 1.8 g (d) oil = 2 g, polymer = 3.6 g (e) oil = 2 g, polymer = 5.4 g (f) oil = 2 g, polymer = 7.2 g.

...out in triplicate. To maintain a constant volume, 5 ml hexane was returned to the container.

2.7 Scanning Electron Microscopy Study

The samples were deposited on a brass holder and sputtered with gold. Surface characteristics of the microcapsules were studied at room temperature using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 15 kv.

2.8 Fourier Transform Infrared (FTIR) Study

The FT-IR spectra were recorded in a Perkin-Elmer RXI FT-IR spectrometer in the range 4000–400 cm$^{-1}$. Gelatin A, sodium alginate, complex of Gelatin A-sodium alginate, olive oil and olive oil loaded microcapsules were each separately finely grounded with KBr and FTIR spectra were recorded.

2.9 Thermal Property Study

Thermal properties of gelatin-alginate neat coacervate complex, crosslinked coacervate complex and microcapsules prepared by using different amounts of crosslinker were recorded by employing a thermogravimetric analyzer (Mettler Toledo) in the temperature range of 30°C to 750°C at a heating rate of 20°C/min in a nitrogen atmosphere.
3 Results and Discussion

The ratio between the gelatin A and sodium alginate was optimized by measuring the coacervate yield (%) and turbidity. Solutions of sodium alginate (0.5% w/v) and gelatin A (0.5% w/v) were prepared in buffer (pH 3.5). Both solutions were mixed in different proportions to make 45 ml. The mixtures were incubated at 40°C for 24 h, and turbidity was measured. The supernatant solution was separated. Coacervate yield (%) of the precipitate and turbidity were...
measured. Each measurement was done in triplicate and the results reported were the average values.

Solutions of gelatin A (0.5% w/v) and sodium alginate (0.5% w/v) were prepared in DDI. Both the solutions were mixed in a definite ratio. The pH of the mixing solution was varied from 2.5–5.0 by using 2.5% glacial acetic acid. Maximum turbidity and maximum yield (%) were found to appear at pH 3.5–3.8. Therefore, all the microencapsulation reactions were carried out at the pH of 3.75.

### 3.1 Turbidity and Coacervate Yield

To optimize the ratio at which maximum coacervation occurred between gelatin A and sodium alginate turbidity measurements were carried out. Turbidity of the supernatant solutions of the mixtures of gelatin A (0.5% w/v) and sodium alginate (0.5% w/v) at different ratios were measured to study the phase separation behavior of gelatin-sodium alginate mixture. The plot of turbidity (NTU) against percentage of gelatin is shown in Figure 2(a). The turbidity increased initially, decreased sharply at a certain point and then again increased slightly. The turbidity of the supernatant dropped sharply after reaching the maximum when the % of gelatin in the mixture was 77.77% i.e. when the sodium alginate to gelatin A ratio was 1:3.5. At this percentage of gelatin, both the polymers probably reacted maximum to form an insoluble complex. The percentage of polymer at this stage in the supernatant would be minimum, which in turn would develop the lowest turbidity. The observed slight higher turbidity at the latter stage might be due to the presence of unreacted polymers in the supernatant.

The plot of coacervate yield (%) against % of gelatin is presented Figure 2(b). With the increase in the percentage of gelatin A coacervate yield (%) increased, reached maximum value and then decreased. The maximum yield was obtained when the percentage of gelatin in the mixture was 77.77%. This could be explained by the fact that at this percentage of gelatin the interaction between the two polymers was maximum and thereby produced maximum coacervate yield.

### 3.2 Effect of Variation of pH

The effect of variation of pH on coacervate yield (%) was measured by taking sodium alginate-gelatin solution (1:3.5) at different pH (2.0–5.0). The plot of pH against coacervate yield (%) was shown in Figure 3. The coacervate yield (%) was found to be the highest at pH 3.75. This implied that the coacervation between the two polymers was highest at this pH and this could be explained as before.

### 3.3 Scanning Electron Microscopy Study

Scanning electron microscope photographs of neat sodium alginate-gelatin complex and olive oil loaded microcapsules were presented in Figures 4 and 5. SEM Photographs neat sodium alginate + gelatin complex without oil loading (Fig. 4a) appeared as a sheet-like structure. The photograph of microcapsules with oil loading but without crosslinker glutaraldehyde appeared agglomerated with no
definite shaped particles. In contrast, the olive oil loaded and crosslinked samples (Fig. 4 c, d) with medium polymer concentration were having free flowing spherical shape. With the increase of the amount of polymer concentration (Fig. 4 b, c), the size of the microcapsules increased. This might be due to the increase of the thickness of the wall of the microcapsules. Similar observations were reported in the literature while studying the particle size of oil loaded gelatin-carrageenan microcapsules (33) and polyurea microcapsules by interfacial polymerisation of polyisocyanates (35). However, in this case with higher polymer concentration the particles appeared again agglomerated (Fig. 4e) and not controlled properly. Particles become needle shaped (Fig. 4f) if polymer concentration was increased beyond this, and might be due to improper control of the reaction because of high viscosity.

Again, with the increase of olive oil loading, keeping polymer concentration similar, the size of the microcapsules becomes relatively larger (Fig. 5a–d). This might be due to an increase of the droplet size of the olive oil with its higher amount. Another reason could be the effect of the drying since drying removed water and not the oil from the microcapsules. So, there occurred less shrinkage for heavily oil-loaded microcapsules resulting in larger sized microcapsules. However, still higher olive oil loading made the microcapsules surface sticky and bursting compared to the low oil loading (Fig. 5 e). At such oil loads the dried microspheres were oily, while those produced at lower oil loads were dry and powdery. Similar observations were found by Chan et al. (27) while studying oil microencapsulation in sodium alginate. The non-ionic surface active emulsifying agent tween-80 played an important role in reducing the surface tension and allowed the stabilization of a greater interfacial surface area, leading to smaller particle sized microcapsules (Fig. 5f–h). Prasertmanakit et al. observed a similar type of observation while studying the effect of surfactant in ethyl cellulose microcapsules (36).

### 3.4 Effect of Variation of Oil Loading

With the increase in oil loading, the encapsulation efficiency, the release rate and % oil content were found to increase throughout the range of oil concentration studied. Table 1 and Figure 6 revealed the effect oil loading variation on oil content, encapsulation efficiency and release rate. Development of larger oil droplets at higher oil load resulted increased encapsulation efficiency. However, as the amount of polymer was fixed, therefore, the polymers would encapsulate all the large oil droplets resulting in a decrease of thickness of the capsule wall and hence, release rate increased. With the decrease in wall thickness, diffusional path for the oil release became short (33), which resulted in an increase in release rate. With an increase in percent oil load, the oil content (%) increased. The encapsulating polymer is sufficient to encapsulate all the oil droplets at very low oil load. But, the number of oil droplets in the microcapsule increased with the increase in oil load (%), which resulted in an increase in oil content. The size and surface characteristics of the microcapsules were found to change as oil content (%) varies as revealed by SEM study (Fig. 5a–e).

### 3.5 Effect of Variation of Cross-Linker Concentration

Crosslinking with glutaraldehyde played an important role in hardening, making the microcapsule wall compact and hence, in retarding the release rate (Fig. 7). The effect of variation of cross-linker concentration on oil loading (%), oil content (%) and encapsulation efficiency (%) is shown in Table 1. With an increase in concentration of glutaraldehyde, oil loading and oil content (%) decreased, but encapsulation efficiency was increased slightly. This increase in encapsulation efficiency was due to the oil retention capacity of the microcapsules due to formation of crosslinking. The release rate of oil was found to decrease as the % of glutaraldehyde increased. The increasing compactness of the microcapsule wall with the increase in degree of crosslinking resulted in the decrease of diffusion rate through the microcapsule wall.

![Fig. 7. Effect of variation of crosslinker concentration on release profile](image)

### Fig. 8. Effect of variation of polymer concentration on release profile

- (a) polymer = 1.8 gm, crosslinker = 1.25 mmol, oil = 4.0 g
- (b) polymer = 3.6 gm, crosslinker = 2.5 mmol, oil = 4.0 g
- (c) polymer = 5.4 gm, crosslinker = 1.25 mmol, oil = 4.0 g.
3.6 Effect of Variation of Polymer Concentration
The effect of variation of total polymer concentration on oil loading, oil content and encapsulation efficiency is shown in Table 1. Both oil loading (%) and oil content (%) decreased with the increase in total polymer content but encapsulation efficiency increased. The increase in encapsulation efficiency was due to the fact that with the increase in polymer content, it would be sufficiently available to encapsulate the oil vesicles. But, the excess polymer after complete encapsulation enhanced the thickness of the microcapsule resulting in the decreased release rate which was shown in Fig. 8. Similar findings were cited in the literature (32–34).

3.7 Fourier Transform Infrared (FTIR) Study
Sodium alginate has free carboxyl group that imparts negative charge to these molecules. Gelatin has positive charge at acidic pH due to the presence of amino groups. During complex coacervation, carboxyl groups in polysaccharides interact with amino groups in protein to form a complex

Fig. 9. FTIR spectra of (a) gelatin A, (b) sodium alginate, (c) gelatin A–sodium alginate complex, (d) olive oil, (e) olive oil loaded microcapsules.
that contains amide linkages (29). 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. 9a) revealed the presence of characteristic functional group at 3444 cm\(^{-1}\) for amino group. In the spectrum of gelatin the other notable peaks observed were at 2925 cm\(^{-1}\) (C–H stretching of alkenes), 2846 cm\(^{-1}\) (C–H stretching of alkanes), 1647 cm\(^{-1}\) (amide-I, CO and CN stretching) and 1530 cm\(^{-1}\) (amide-II). Other peaks observed were at 1160 cm\(^{-1}\), 1028 cm\(^{-1}\) due to, C–O stretching of carboxylic acid and C–N stretching of amines, respectively. FTIR spectrum of alginate (fig.9b) showed stretching frequency for carboxylic acid group at 2927 cm\(^{-1}\). In the spectrum of sodium alginate, the following peaks were observed at 3443 cm\(^{-1}\), 2928 cm\(^{-1}\), 1615 cm\(^{-1}\), 1096 cm\(^{-1}\), 1029 cm\(^{-1}\), which were assigned due to alcoholic O–H stretching, O–H stretching for carboxylic group, carboxylate salt asymmetric stretch and C–O stretching of ether, respectively. The peaks of free amino groups that present in gelatin disappeared in gelatin-alginate complex coacervate (Fig. 9c). A characteristic peak for amide in the region of 1500–1650 cm\(^{-1}\) appeared in the complex coacervate (1637 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 slight shift of the peak of amide I from 1647 cm\(^{-1}\) to 1637 cm\(^{-1}\) observed in the complex of gelatin and sodium alginate also indicated that the negatively groups of alginate might associate with positively charged gelatin. A similar type of observation was reported by Muyona et al. (37) and Pranoto et al. (38). The FTIR spectrum of olive oil (Fig. 9d) showed the following characteristic peaks at 3473 cm\(^{-1}\), 2924 cm\(^{-1}\), 2854 cm\(^{-1}\), 1746 cm\(^{-1}\), 1657 cm\(^{-1}\), 1463 cm\(^{-1}\), 1417 cm\(^{-1}\), 1377 cm\(^{-1}\) due to O–H symmetric stretch, C–H alkenes stretching, C–H alkanes stretching, O–H stretch of carboxylic acid, C=O stretching, C=C stretch of alkenes, -CH\(_2\) bending and -CH\(_3\) bending, respectively. The FTIR spectrum of the oil loaded microcapsules (Fig. 9e) showed the characteristic peaks of olive oil and confirmed successful encapsulation oil and absence of any significant interaction between oil and polymer.

### 3.8 Thermal Property Study

Thermogravimetric curves of neat gelatin-alginate complex, crosslinked gelatin-alginate complex and microcapsules prepared with different amount of glutaraldehyde were shown in Figure 10. The major weight loss for the neat coacervate was observed at around 330°C, while the corresponding temperature for crosslinked coacervate was at around 350°C. The presence of crosslinker increased the thermal stability of the coacervate. TGA curves for gelatin-alginate complex and microcapsules were quite similar to each other except that thermal stability was improved by crosslinking. Temperature of decomposition values for crosslinked microcapsules were found to be higher than those of the complex (i.e., without crosslinker) and temperature of decomposition values increase with the increase in the amount of crosslinker. The increasing trend of the temperature of decomposition values might be due to the decreasing chance of elimination of small molecules like CO\(_2\), CO etc. with the formation of crosslinking, which acted as an infusible support and provided thermal resistance to the microparticles. Olive oil release studies also supported the above observation. Moreover, the TGA thermograms of the neat crosslinked coacervate and the oil loaded microcapsules showed very little difference indicating the absence of very strong interactive force between the oil and the complex.

### 4 Conclusions

Olive oil could be encapsulated efficiently using gelatin A-sodium alginate complex using glutaraldehyde as crosslinker. Maximum coacervation occurred at 3.5:1 gelatin to sodium alginate ratio and at pH of 3.75. The encapsulation efficiency was found to increase with the increase in the concentration of olive oil, glutaraldehyde and polymer. SEM study revealed that the size of the microcapsules were increased as the amount of olive oil and polymer concentration increased but the size decreased as the amount of tween 80 increased. FTIR and TGA study did not exhibit any remarkable interaction between olive oil and gelatin-sodium alginate complex.
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