ISSN 0974 - 9373

Vol. 15(2011) Special Issue 2 **Journal of International Academy of Physical Sciences** pp. 455-476

Study of Complex Coacervation of Chitosan with Sodium Carboxymethyl Cellulose: Microencapsulation of Neem Seed Oil and Isoniazid*

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(Received June 14, 2011)

Abstract: Microparticles containing natural liquid pesticide neem (*Azadirachta Indica A.Juss*.) seed oil (NSO) and a solid antitubercular drug, isoniazid were prepared using complex coacervation technique and employing chitosan and sodium carboxymethyl cellulose polyelectrolyte complex. Turbidity measurement was carried out to evaluate the pH and ratio of the two polymers that produced highest yield. Effective coacervation was realized over the pH range of 2.5-3.5 using the chitosan and sodium carboxymethyl cellulose ratio of 1:2.33.The encapsulation efficiency of NSO and Isoniazid were dependent on the amount of crosslinker, oil/drug loading and polymer concentration. Scanning electron micrographs showed the formation of free flowing spherical microparticles in case of isoniazid loading and a bit agglomerated in case of neem seed oil loading. The size of microparticles tends to increase with the increase of the concentration of the polymer. Thermogravimetric analysis showed the improvement of thermal stability with crosslinking. Fourier Transform Infrared Spectroscopy study showed that there was no significant interaction between NSO/drug and chitosan and sodium carboxymethyl cellulose complex.
Keywords: Microparticles; Chitosan; Sodium carboxymethyl cellulose; Complex coacervation, Crosslinking.

1. Introduction

Recently a significant improvement has been experienced within the agricultural and pharmaceutical sectors regarding such environment-friendly technologies that mainly target the production of healthy, nutritious, quality foodstuff as well as pharmaceutical products by taking environmental characteristics into account, and adapting to them. To achieve such goals, scientists today are more than ever before being challenged to provide environmentally benign, more economical and more efficient products for

*Paper presented in CONIAPS XIII at UPES, Dehradun during June 14-16, 2011.

the health and well being of mankind. Controlled delivery by microencapsulation technologies has emerged as an approach with promise not only to utilize resources in the maximum efficient way but also for the prevention of pollution. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix, to form small capsules[1-3] and build a barrier between the component in the particle and the environment. The core may be composed of just one or several ingredients and the wall may be single, double or multiple layered.

Pest management is an integral part of modern agriculture and its efficacy can, in many cases, make the difference between success and failure of the grower[4]. The damage caused by pests is estimated to be between 35 and 40% of all crops grown[5]. The use of synthetic pesticides has undoubtedly resulted in increased crop production. However, these chemicals are hazardous both to man and the environment. Natural pesticides are ecofriendly, safe and less toxic compared to synthetic pesticides. In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries but can also play much greater role in the production and post harvest protection of food in developing countries[6]. Neem seed oil (NSO) extracted from the seed of plant *Azadirachta Indica A. Juss.* has already proved its potential as effective natural pesticide. But due to the liquid nature the application of NSO to the soil is limited. For utilization of this potent natural biopesticide to soil, it needs solid form which can be achieved by microencapsulation technique. Moreover, azadirachtin, the key component of NSO is rapidly degraded by sunlight[6]. Thus, Microencapsulation of NSO not only gives it a solid form but also protects it from sunlight.

Isoniazid is widely used in the chemotherapy of tuberculosis. Tuberculosis is one of the various diseases that have afflicted the human race for centuries. Approximately one-third of the world population is infected with *Mycobacterium tuberculosis* (TB), resulting in more than eight million new cases and two million deaths annually[7]. While potentially curative treatments have been available for almost half a century, TB remains the leading cause of preventable deaths and hence continues to present a formidable challenge as a global health problem. One of the major problems is non-compliance to prescribed regimens, primarily because effective chemotherapy of TB involves the daily administration of one or more drugs for a period of 6 months or longer. Clinical management of the disease is limited because of toxic side effects of drugs, degradation of drugs before reaching their target site, low permeability and poor patient compliance[8].

Thus, the drawbacks of conventional chemotherapy necessitate the development of a delivery or carrier system to release drugs slowly over extended time periods, which would also allow reduction in frequency and dosing numbers.

Chitosan is a hydrophilic cationic polyelectrolyte obtained by alkaline *N*-deacetylation of chitin. Chitin is the most abundant natural polymer next to cellulose and is obtained from crab and shrimp shells[9]. Chitosan has been broadly evaluated by the industries due to its biocompatibility and its potential use in controlled release systems as membranes, tablets and microspheres. Sodium carboxymethyl cellulose (SCMC) is an anionic derivative of cellulose. They are, thus, expected to interact and form polyelectrolyte complex under controlled conditions.

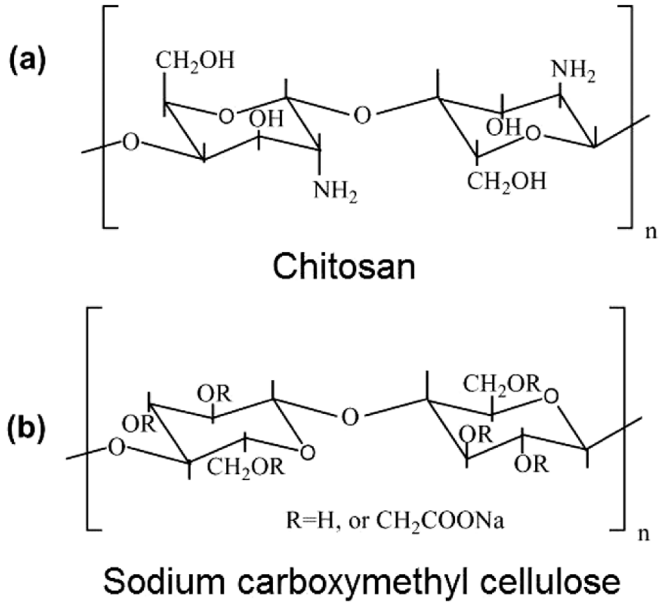

Fig.1. Chemical structure of (a) Chitosan and (b) SCMC

Present research work aims at microencapsulation of the potent natural pesticide NSO and an antitubercular drug, isoniazid. This work also aims at to study the complex coacervation of chitosan with sodium carboxymethyl cellulose and microencapsulation of the two active agents in the polyelectrolyte complex system. Besides, this process involves use of water as a solvent and a vegetable oil (Sunflower oil) as emulsion medium to eliminate the organic solvent particularly the most popularly used paraffin oil[10-12]. Efforts are also made to characterize and study the encapsulation efficiency and release behaviour of microparticles.

2. Materials and Methods

2.1. Materials

Chitosan, low molecular weight was purchased from Sigma-Aldrich Inc. (USA). SCMC (medium viscosity) was purchased from Rankem (India).Glacial acetic acid (E.Merck, India), tween 80 (E.Merck, India) and glutaraldehyde 25% w/v (E. Merck, Germany) were used as such received. Cold pressed NSO was obtained from Ozone Biotech., Faridabad, India. Isoniazid was purchased from Sigma-Aldrich Inc. (USA). Edible grade refined sunflower oil was purchased from local market. DDI (double-distilled deionised) water was used throughout the study. Other reagents used were of analytical grade.

2.2. Microencapsulation

2.2.1. Polyelectrolyte Complexation Conditions

Polyelectrolyte complex formation between two oppositely charged polymers depends on several parameters such as pH of the polymer olutions, ratio between the polymers, temperature etc. The optimal conditions for the formation of polyelectrolyte complex of chitosan/SCMC were evaluated by monitoring the absorbance of the supernatant of the mixture of the two polymers at various ratios the polymers and at various pH conditions. The optimum ratio of Chitosan to SCMC and pH range at which maximum complexation (judged by the turbidity or absorbance) occurred were 1.0: 2.33 and 2.5-3.5 respectively. All the successive experiments were performed at this optimal pH and polymer ratio.

2.2.2. Microencapsulation Procedure

2.2.2.1. Microencapsulation of NSO

A known amount of (100ml) 0.5-3.0 % (w/v) of SCMC solution was taken in a beaker. This polymer solution was stirred by mechanical stirrer under high agitation at 60 ± 1^0C. This temperature was maintained throughout the experiment. To this, NSO (2-8g) was added under high agitation to form an emulsion. A known amount of (43ml) chitosan solution of 1-3% (w/v) was added to the beaker drop wise to attain complete phase separation. However, the weight ratio of chitosan to SCMC was maintained at 1: 2.33 during all the experiments. At this ratio, interaction between chitosan and SCMC took place completely as judged by the yield and turbidity measurements. The pH of the mixture was then brought down to 3.0-3.5 by adding glacial acetic acid solution. At pH=2.5-3.5, the yield was maximum as judged by yield and turbidity measurements. The beaker containing the microparticles was left to rest at this temperature for approximately 15

minutes. The system was then brought to 5-10^{0}C to harden the microcapsules. The cross linking of the polymer was achieved by slow addition of certain amount of glutaraldehyde solution (5.0-20.0 mmol). The temperature of the beaker was then raised to 45°C and stirring was continued for another 3-4 hrs to complete the crosslinking reaction. The beaker was then cooled to room temperature slowly while stirring. The microparticles were filtered through 300-mesh nylon cloth, washed with 0.1% Tween 80 surfactant solution to remove oil, if any, adhered to the surface of microparticles. This was further washed with distilled water, freeze-dried and stored inside a refrigerator in a glass ampule.

2.2.2.2. Microencapsulation of Isoniazid

To a beaker containing a known amount of (150 ml) sunflower oil, 30 ml of SCMC solution (0.5-3% w/v) was added, under stirring condition at 60±1^{0}C to form an emulsion. (0-1) g of the tween 80, dissolved in 10 ml of water was added to the beaker to stabilize the emulsion. A known amount of (13 ml) chitosan solution of same concentration (0.5-3% w/v) was added to the beaker drop wise to attain complete phase separation. However, the weight ratio of SCMC to Chitosan was maintained at 1:2.33 during all the experiments. The pH of the mixture was then brought down to 3.0-3.5 by adding glacial acetic acid solution. The beaker containing the microparticles was left to rest at this temperature for approximately 15 minutes. The system was then brought to 5-10^{0}C to harden the microparticles. Glutaraldehyde (4.375-17.50 mmol/g of polymer) solution was added slowly for crosslinking. The temperature of the beaker was then allowed to rise to 45 ^{0}C and stirring was continued for another 3 hrs to complete the crosslinking reaction. The microparticles were filtered through 300-mesh nylon cloth, washed with acetone to remove oil, if any, adhered to the surface of microparticles. This was further washed with distilled water, and freeze-dried. The dried microparticles were then dipped in isoniazid solution (10%, w/v) for 48 hours, filtered through 300-mesh nylon cloth, and quickly washed with water to remove the surface adhered isoniazid. The isoniazid-encapsulated microparticles were again freeze-dried and stored in a glass bottle in refrigerator.

2.3 Measurement of Turbidity

The mixing of chitosan and SCMC in different ratios would produce solutions of different turbidity. The optimal ratio at which complete phase separation occurred between chitosan and SCMC was the point where the polymer mixture solution would have the maximum turbidity i.e. the supernatant would have minimum turbidity. The change in absorbance due to

turbidity was monitored at a particular wavelength employing UV spectrophotometer. Polymer in the supernatant solution would be either negligible or absent when the interaction between chitosan and SCMC would be maximum. At this stage, the turbidity of the supernatant would be close or similar to the solvent turbidity. Similarly, at a certain chitosan and SCMC ratio, pH of the mixture solution was changed from 2.0-5.5 by addition of acetic acid and change in absorbance due to turbidity was monitored.

2.4 Calibration Curves of Oil and Isoniazid

A calibration curve is required for the determination of release rate of oil from the microcapsules. It was found that 0.1 gm of oil could be easily dissolved in 100 ml of water containing 0.1 g Tween 80. A known concentration of NSO in DDI water containing 0.1% Tween 80 was scanned in the range of 200- 400 nm by using UV visible spectrophotometer. For NSO having concentration in the range 0.001 to 0.08 g /100ml, a prominent peak at 254 nm was noticed. The absorbance values at 254nm obtained with the respective concentrations were recorded and plotted. Similarly, for isoniazid, a known concentration of isoniazid in DDI water was scanned in the range of 200-500 nm by using UV visible spectrophotometer. For isoniazid having concentration in the range 0.001 to 0.01 gm/100ml, a prominent peak at 261 nm was noticed. The absorbance values at 261nm obtained with the respective concentrations were recorded and plotted. From the calibration curves, the unknown concentrations of the active agents (oil/isoniazid) were obtained by knowing the absorbance value.

2.5. Determination of Oil Loading, Oil Encapsulation Efficiency and Isoniazid Loading Efficiency

A known amount of accurately weighed NSO microcapsules was grounded in a mortar, transferred with precaution to a volumetric flask containing a known amount of 0.1% (w/v) aqueous Tween 80 solution and kept for overnight with continuous stirring. The encapsulation efficiency (%), oil content (%) and oil loading (%) were calculated by using the calibration curve and the following formulae[13].

Encapsulation efficiency (%) = $(w_1 / w_2) \times 100$,

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

where, w = weight of microcapsules

w_1 = actual amount of oil encapsulated in a known amount of microcapsules

w_2 = amount of oil introduced in the same amount of microcapsules

w_3 = total amount of polymer used including crosslinker

In case of isoniazid loaded microparticles, they were grounded in a mortar, transferred with precaution to a volumetric flask containing 100ml of water (having pH=7.4, maintained by phosphate buffer solution) and kept for overnight with continuous stirring to dissolve the isoniazid in the microparticles. The solution was collected and the isoniazid inside the microparticles was determined employing UV spectrophotometer. The loading efficiency (%), was calculated by using the calibration curve and the following formulae

Loading efficiency (%) = $w_1 / w_2 \times 100$,

where, w_1 = amount of isoniazid encapsulated in a known amount of microparticles, w_2 = weight of microparticles.

2.6. Release Behaviour Studies

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

For isoniazid release studies from the isoniazid-encapsulated microparticles a known quantity of microparticles was taken into a known volume 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 261 nm for the determination of cumulative amount of drug release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5 ml of the solution having same pH 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 microparticles were studied at room temperature using scanning electron microscope (model JEOL, JSM-6390) at an accelerated voltage of 5-15kv.

2.8. Fourier Transform Infrared (FTIR) Study

FTIR spectra were recorded using KBr pellet in a Nicolet (model Impact-410) spectrophotometer. Chitosan, SCMC, polyelectrolyte complex of chitosan-SCMC at different ratios, isoniazid, isoniazid loaded microparticles, NSO and NSO loaded microparticles were each separately finely grounded with KBr and FTIR spectra were recorded in the range of 4000-400cm^{-1}.

2.9. Thermal Property Study

Thermal properties of chitosan, SCMC, polyelectrolyte complex of chitosan-SCMC at different crosslinking, isoniazid, isoniazid loaded microparticles, NSO and NSO loaded microparticles were evaluated by employing a thermogravimetric analyzer at a heating rate of 10^0C/min up to 750^0C. All the study were done under nitrogen atmosphere.

3. Results and Discussion

3.1. Effect of Variation of Ratio between the Polymers

The ratio between the chitosan and SCMC was optimised by measuring the turbidity of the supernatant. Solutions of SCMC (0.5% w/v) and Chitosan (0.5% w/v) were prepared in acetic acid/sodium acetate buffer (pH 3.5). Both solutions were mixed in different proportions to make 45ml. The mixtures were incubated at 40^0C for 24 hours and the supernatant solution was separated.

Solutions of chitosan (0.5% w/v), SCMC (0.5% w/v) and the mixture of both at different ratios were scanned in the range 200-800 nm employing UV spectrophotometer. Solutions of both the polymers showed low absorbance at 360 nm while the mixture showed more absorbance at this wavelength. In all the subsequent experiments, therefore, this wave length was used to scan and study the phase separation behaviour of chitosan-SCMC mixture.

The plot of absorbance (%) against percentage of chitosan in the mixture was presented in fig.2 The absorbance increased initially, reached maximum, decreased sharply after this point and again increase slightly. The maximum absorbance and sharp decrease of absorbance occurred when the % of chitosan in the mixture was 30% and 33.33% respectively. The maximum turbidity developed when the interaction between chitosan and SCMC tends to be maximum and after reaching this point the polyelectrolyte complex formed precipitates very fast (within five minutes).

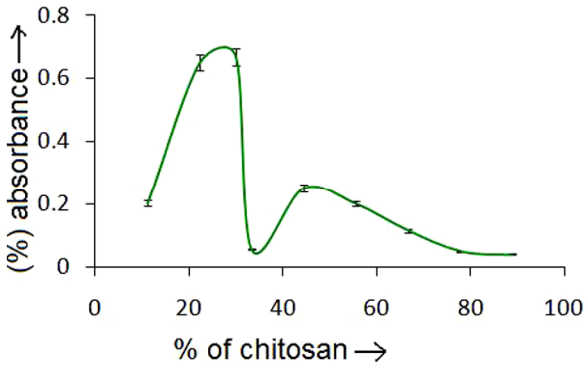

Fig.2. Change in absorbance of the supernatant with the percentage of chitosan in the chitosan-SCMC polymer mixture

Due to this, the absorbance of the supernatant decreases sharply. The observed higher absorbance at the latter stage might be due to the presence of unreacted chitosan in the supernatant. The finding was further confirmed by monitoring the absorbance for dilute solutions having very low polymer concentrations and checking the coacervate yield (%). Yield (%) was found highest at chitosan-SCMC ratio of 1:2.33 (i.e.33.33% of chitosan in the mixture).

Fig.3. Optical micrographs of appearance of turbidity at chitosan-SCMC ratio of 1:2.33 and pH 3.5. (A) immediately after reaching the ratio and stopping of stirring ;(A1) after 2 minutes of reaching the ratio and stopping of stirring; (A2) after 5 minutes of reaching the ratio and stopping of stirring. At low stirring speed and high polymer concentration the complex precipitates in all the cases (B) immediately (B1) after 2 minutes and (B2) after 5 minutes

3.2. Effect of Variation of pH

When the pH of the mixture solution of chitosan and SCMC was below 2.0, it was observed that no precipitates were formed and above pH 5.5 of the mixture formation of a gel like product was observed. Above pH 6.5,

chitosan is insoluble. Therefore, study for effect of variation of pH was carried out in the pH range of 2.5-5.5.

Solutions of chitosan (0.5% w/v) and SCMC (0.5% w/v) were prepared in 0.1% (v/v) acetic acid and DDI respectively. Both the solutions were mixed in the ratio of 1: 2.33. The pH of the mixing solution was varied from 2.0-5.5 by using glacial acetic acid. The mixtures were incubated at 40^0C for 24 hours and the supernatant solution was separated.

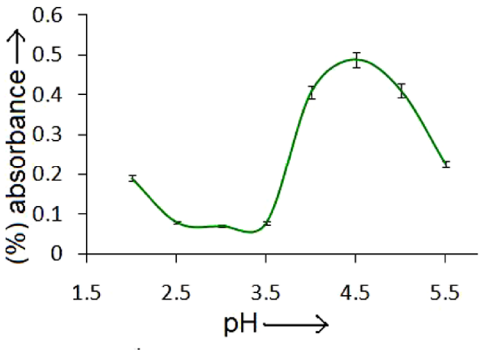

Fig.4. The effect of variation of pH on turbidity

The effect of variation of pH on turbidity was measured by checking absorbance (at 360 nm) of chitosan-SCMC solution (1:2.33) at different pH (2.5-5.5). The turbidity was found to increase up to pH 2.5. Turbidity is minimum after pH 2.5 to till pH 2.5 due to clear precipation of the complex. Beyond that it again increased. This implied that the coacervation between the two polymers was highest at the pH of 2.5-3.5 (Fig.4). The explanation for this was similar to that of given earlier. The finding was further confirmed by monitoring the absorbance for dilute solutions having very low polymer concentrations and checking the coacervate yield (%). Yield (%) was found highest at pH 2.5-3.5.

3.3. Scanning Electron Microscopy Study

SEM photographs of neat chitosan-SCMC complex and NSO loaded microparticles are presented in fig 5. Photographs of neat chitosan-SCMC complex (fig 5a) appeared agglomerated with no definite structure. In contrast, the NSO loaded samples (fig.5 b,c,d,e,f,g,h) were having free flowing spherical shape. With the decrease of the amount of polymer concentration (fig.5.b to c,e,g), the size of the microparticles decreased. This might be due to the decrease of the thickness of the wall of the microparticles. Again with the use of surfactant tween 80, the size of the microparticles decreased due to the emulsifying capacity of the surfactant

tween 80 (Fig.5 pairs c and d; e and f; g and h compared). Similar observations were reported in the literature while studying the particle size of polyurea microcapsules by interfacial polymerisation of polyisocyanates[14].

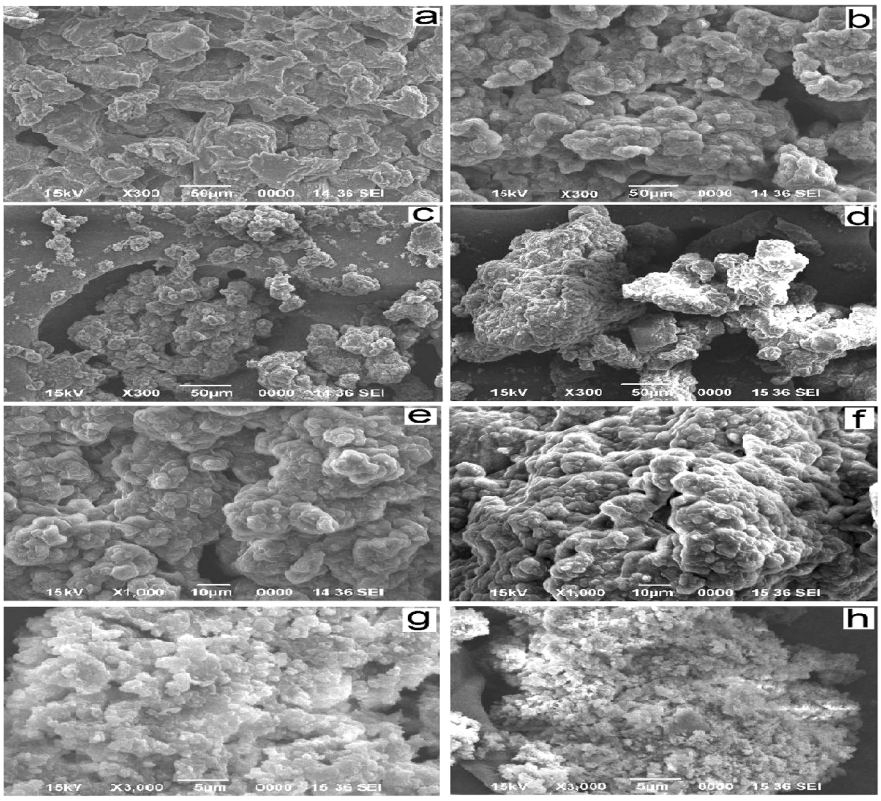

Fig.5. Scanning electron micrographs of (a) chitosan-SCMC complex (b) Totalpolymer=4.29g,NSO=5.0g,glutaraldehyde=12.5mmol(c)Polymer=2.86g,NSO=5.0g,glutaraldehyde=12.5mmol (d) Polymer=2.86g,NSO=5.0g, glutaraldehyde=12.5mmol, tween 80= 0.5g (e) Polymer=1.43g,NSO=5.0g, glutaraldehyde=12.5mmol (f) Polymer=1.43g,NSO=5.0g, glutaraldehyde=12.5g, tween 80=0.5g (g) Polymer=0.715g,NSO=5.0g, glutaraldehyde=12.5mmol, (h) Polymer=0.715g,NSO=5.0g, glutaraldehyde=12.5mmol, Tween 80=0.5g

Similar type of results was obtained for microparticles prepared in sunflower oil as medium using tween 80 as emulsifier. Tween 80 had played a profound effect on the formation of these chitosan-SCMC microparticles in sunflower oil. Without the surfactant, a matrix gel like product was formed (fig.6a). But different types of microparticles ranging from agglomerated to free flowing were formed on addition of varying amount of surfactant (fig.6.b,c). Smaller sized microparticles were formed with the increase of

amount of tween-80. 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.

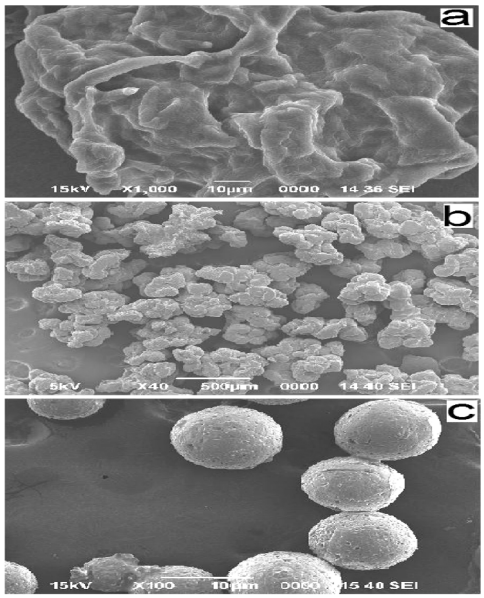

Fig.6. Scanning electron micrographs of microparticles (a) without tween 80 (b) Total polymer=1.43g,glutaraldehyde=6.50mmol/g of polymer, tween 80=0.50g (c) Polymer=1.43g, glutaraldehyde=6.50mmol/g of polymer, tween 80=1.0g

3.4. Effect of Variation of Oil Loading

The effect of variation of oil loading on encapsulation efficiency and release rate is shown in the Table1 and Fig 7.

With the increase in oil loading, the encapsulation efficiency and the release rate were found to increase throughout the range of oil concentration studied. At low oil load, the dispersion force of the stirrer was more efficient resulting in the generation of smaller oil droplets. The polymer present in the mixture was enough to encapsulate these droplets. The dispersion force became progressively difficult as the oil load increased. This would develop large oil droplets and as a result encapsulation efficiency would increase. As the amount of polymer was fixed, therefore, the polymers would encapsulate all the large oil droplets at the expense of decrease of thickness of microparticle wall. The faster release rate might be due to the decrease of thickness of the capsule wall. With the decrease in wall thickness, diffusional path for the oil release became short[15] which resulted in an increase in release rate.

Table 1: Effect of variation of oil loading, polymer and glutaraldehyde concentration on the behaviour of microcapsules.[chitosan=0.215-1.29 g; SCMC= 0.5-3.0 g; water=143ml; NSO=2.0-8.0 g ; glutaraldehyde= 5.0-20.0 mmol; temperature: 60±1°C]

Sample formulations			Oil load (%)	Encapsulation efficiency (%)
Total polymer (gm)	Glutaraldehyde (mmol)	NSO (gm)		
2.86	12.5	2.0	48.66	72.85±2.51
2.86	12.5	5.0	121.65	82.51±2.57
2.86	12.5	8.0	194.64	93.78±1.65
2.86	5.0	5.0	148.80	78.16±1.24
2.86	20.0	5.0	102.88	94.78±1.76
0.715	5.0	5.0	411.52	69.56±2.48
1.43	5.0	5.0	259.06	75.96±1.35
4.29	5.0	5.0	115.20	95.45±2.88

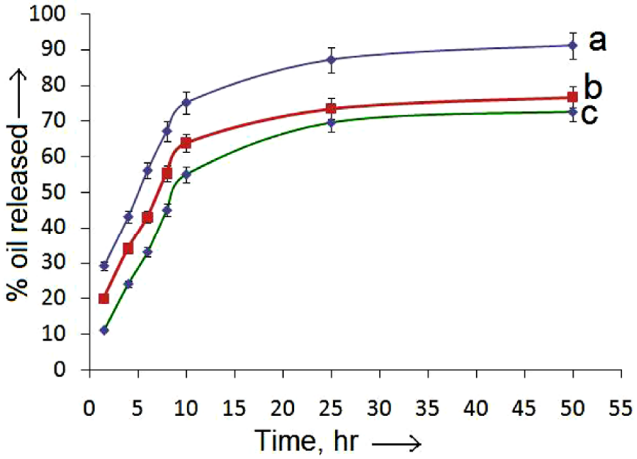

Fig.7. NSO release profile of formulations having (a) Polymer=2.86g, NSO=8.0g,glutaraldehyde=12.5mmol (b) Polymer=2.86g, NSO=5.0g,glutaraldehyde=12.5 mmol (c) Polymer=2.86g, NSO=2.0g,glutaraldehyde=12.5 mmol

3.5. Effect of Variation of Cross-Linker Concentration

The effect of variation of cross-linker concentration on oil loading (%), encapsulation efficiency (%) and release rate is shown in the Table 1 and Fig 8. The trends of oil loading (%) shown in the table were as per expectation. With the increase in glutaraldehyde concentration, oil loading decreased for all but encapsulation efficiency increased. The increase in encapsulation efficiency (%) could be due to the improvement of oil retention capacity of the microcapsules caused by the formation of crosslinking. The crosslinking reaction took place between glutaraldehyde and polyelectrolyte complex of chitosan and SCMC. The release rate of oil was found to decrease as the % of glutaraldehyde increased. The microparticle wall became compact as degree of crosslinking increased. This resulted in the decrease of diffusion rate through the microparticle wall. Similar findings were cited in the literature [16].

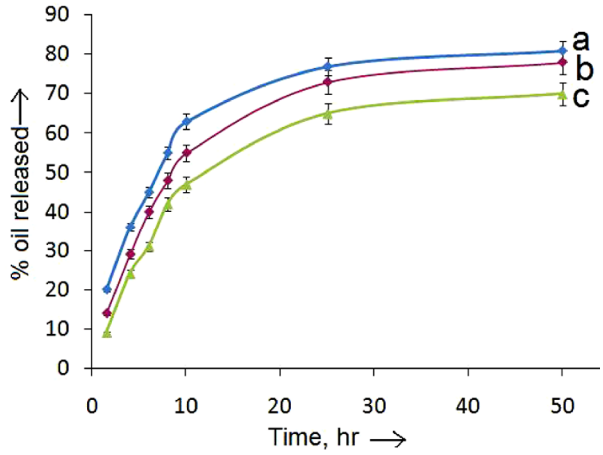

Fig.8. Effect of variation of crosslinker concentration on release profile [(a) Polymer=2.86g, NSO=5.0g, glutaraldehyde= 5.0 mmol (b) Polymer=2.86g, NSO=5.0g,glutaraldehyde=12.5 mmol (c) Polymer=2.86g, NSO=5.0g, glutaraldehyde=20.0 mmol]

The effect of variation of glutaraldehyde concentration on the microparticles prepared in sunflower oil for encapsulation of isoniazid is presented in table 2 and fig.9. An increase in loading efficiency was observed as immersion time increased. This increase in loading efficiency was due to the more diffusion of isoniazid into the microparticles. But with the higher amount of crosslinker in the microparticles, the loading efficiency was lowered. 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 of isoniazid. But, when immersed in similar concentration of isoniazid solution (10%) for a longer period, both high and low crosslinked

microparticles showed almost similar loading efficiency. Longer immersion time allowed the microparticles to become saturate with isoniazid solution.

Table 2. Effect of variation of crosslinker concentration and immersion time on loading efficiency. [Isoniazid=10% (w/v) in DDI, SCMC=1g, Chitosan=0.43g]

Concentration of glutaraldehyde (mmol)/g of polymer	Time of immersion (h)	Loading efficiency (%)
2.25	2	36.0 ± 0.21
2.25	8	44.0 ± 0.56
2.25	20	50.0 ± 0.42
2.25	32	54.0 ± 0.58
6.50	2	31.0 ± 0.61
6.50	8	36.0 ± 0.21
6.50	20	42.25± 0.42
6.50	32	50.25± 0.42
2.25	48	58.26 ± 0.57
6.50	48	56.37 ± 0.28
10.50	48	55.42 ± 0.36

The drug release profile was found to decrease with the increase in the amount of crosslinker in the microparticles. Initially, the release rate was fast and then drug release was slow for the next 48 hours of study. The compact microparticle wall was responsible for the decrease in release rate as explained earlier. Drug release was dependent on pH. For the same crosslinker concentration, at acidic pH (pH=1.2), isoniazid release was less compared to that of at higher pH (pH=7.4). The reason behind the pH dependent release can be explained in that, at low pH condition, the amino groups from chitosan were protonated and the electrostatic interaction of carboxyl groups of SCMC with the amino groups of chitosan was strengthened, resulting in a dense structure [17].

3.6. Effect of variation of polymer concentration

Table 1 shows the results of the effect of variation of total polymer concentration on oil loading and encapsulation efficiency. Oil loading (%) decreased with the increase in total polymer content but encapsulation efficiency increased. With the increase in polymer content, more and more polymer would be available to encapsulate the oil vesicles and thereby efficiency increased. The excess polymer after complete encapsulation would enhance the thickness of the microcapsule. The release profile is shown in fig.10. The release rate was found to decrease with the increase in polymer concentration. The increase in wall thickness of the microcapsules might be responsible for this type of behaviour [16,18].

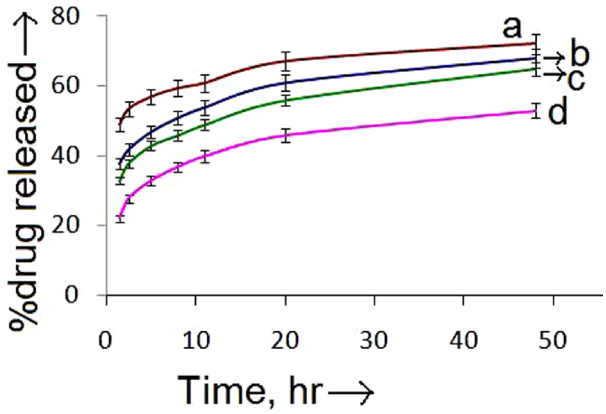

Fig.9. Release profile of isoniazid with the variation of crosslinker concentration and pH [(a) polymer = 1.43 g; crosslinker = 2.25 mmol/g of polymer; pH=7.4, (b) polymer = 1.43 g; crosslinker =2.25 mmol/g of polymer; pH=1.2, (c) polymer =1.43; crosslinker = 6.50 mmol/g of polymer; pH=7.4, (d) polymer=1.43 g; crosslinker = 6.50 mmol/g of polymer; pH=1.2]

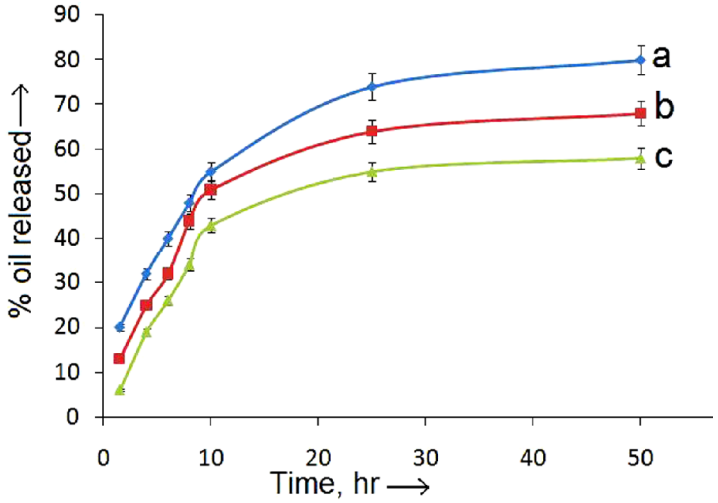

Fig.10. Effect of variation of polymer concentration on release profile [(a) Polymer=0.715g, NSO=5.0g, glutaraldehyde= 5.0 mmol (b) Polymer=2.86g, NSO=5.0g,glutaraldehyde=5.0 mmol (c) Polymer=4.29 g, NSO=5.0g, glutaraldehyde=5.0 mmol]

3.7. Fourier Transform Infrared (FTIR) Study

The FTIR spectrum of SCMC, chitosan, chitosan-SCMC complexes prepared at various chitosan and SCMC ratios are presented in fig.11 and microparticles, NSO, NSO loaded microparticles, isoniazid and isoniazid loaded microparticles are presented in fig.13. SCMC showed absorption bands at 3364 cm^{-1}, 2942 cm^{-1}, 1627 cm^{-1}, 1422 cm^{-1}, 1063 cm^{-1} which were

due to O-H stretching vibration, CH_3 symmetric+ CH_2 assymetric vibration, C-O stretching band for cellulose, CH_3 + CH_2 bending vibration and strong C-O stretching band for ethers. The spectrum of chitosan showed a strong absorption band at 1639.33 cm^{-1} assigned to NH bending. The other notable peaks appeared at 3435, 2920, 1425 and 1384 , 1330 , 1170 , 1075 , and 1030 cm^{-1} , were due to O-H + N-H stretching vibration, CH_3 symmetric + CH_2 asymmetric vibration , CH_3 +CH_2 bending vibration, vibration of C-N group, C-O-C asymmetric vibration, C-O(-C-OH-) vibration , C-O(-CH_2 -OH-) vibration respectively.

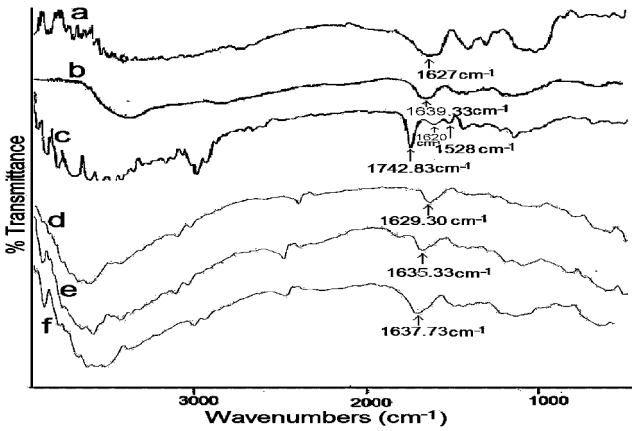

Fig.11: FTIR spectra of (a) SCMC (b) Chitosan c) Chitosan-SCMC complex (at ratio 1:2.33) (d) Chitosan-SCMC complex (at ratio 1:1.5) e) Chitosan-SCMC complex (at ratio 1:0.5) (f) Chitosan-SCMC complex (at ratio 1:0.12)

The characteristic peak of chitosan at 1639.99 cm^{-1} was changed to 1620 cm^{-1} (fig. b, f,e,d,c) gradually with increasing complexation between chitosan and SCMC. A new absorption band peak at 1742.83 cm^{-1} was observed in the chitosan-SCMC complex (fig.c) which was caused by the electrostatic interaction between the –COOH groups of CMC and $–NH_2$ groups of chitosan [17]. Another new and weak absorption band around 1528 cm^{-1} assigned to $–NH_3^+$ groups is observed in the polyelectrolyte complex. Thus $–NH_3^+$ groups in chitosan participate in binding with SCMC probably through its $–COO^-$ groups[19]. The reaction mechanism of complexation and crosslinking is shown in fig.12.

The absorption bands appeared in the spectrum (fig.13) of NSO at 1743 cm^{-1}, 1456 cm^{-1} and 1163 cm^{-1} were due to carbonyl stretching, CH_2 asymmetric deformation and C-C stretching vibration. In the spectrum of isoniazid (fig.13), the carbonyl absorption (amide I band) appeared at 1664 cm^{-1}. The amide II band that occurred at 1555.90 cm^{-1} was due to N-H

bending of the secondary amide group. Moreover, in the spectrum of isoniazid, multiple bands appeared between 1400 cm^{-1} to 668 cm^{-1}. The peaks observed in the spectrum of NSO and isoniazid were appeared in the NSO loaded and isoniazid loaded microparticles respectively. This confirmed the successful encapsulation of NSO and isoniazid in the micrparticles.

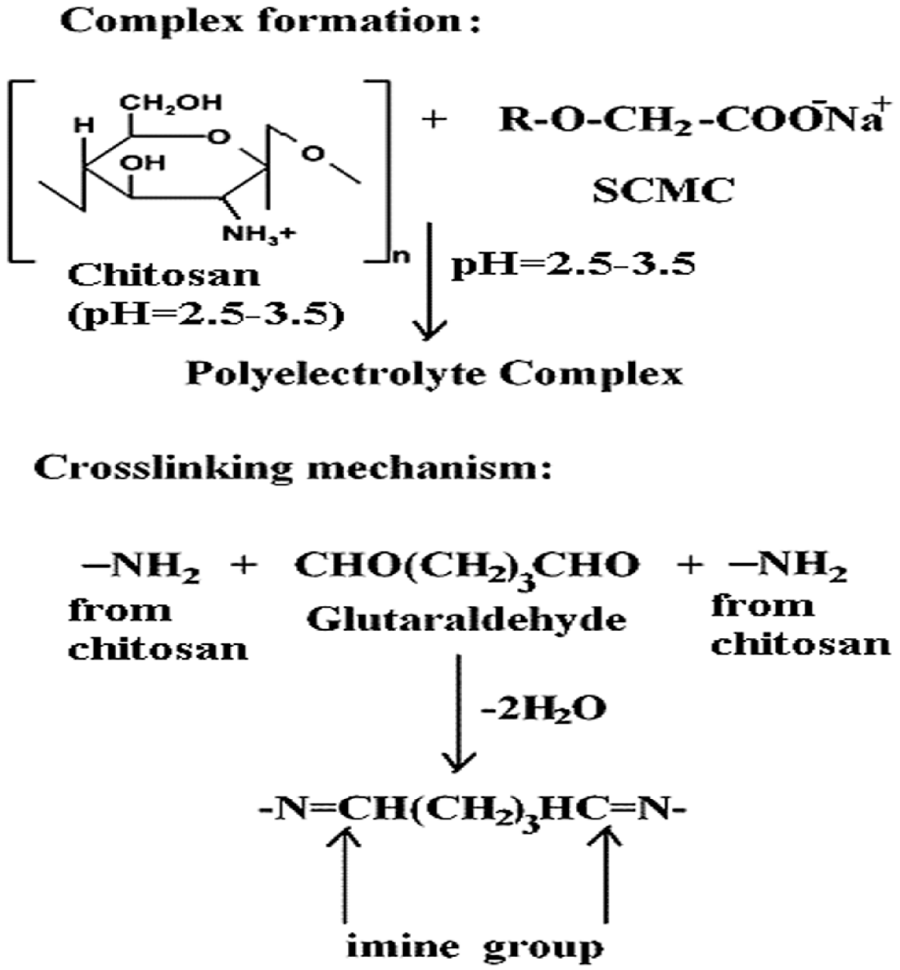

Fig.12: Polyelectrolyte complexation and crosslinking between chitosan, SCMC and glutaraldehyde

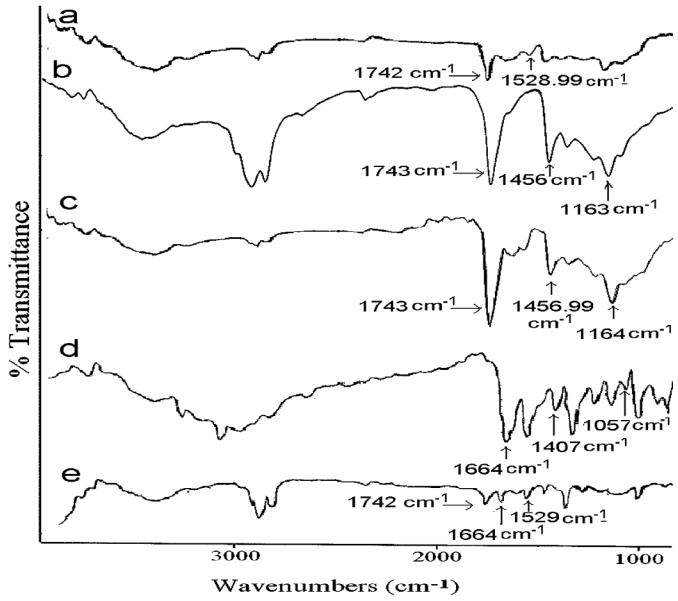

Fig.13. FTIR spectra of a) chitosan-SCMC microparticles without loading b) NSO c) NSO loaded microparticles d) isoniazid e) isoniazid loaded microparticles

3.8. Thermal property study

Fig.14 and fig.15 shows the TGA curves of chitosan, SCMC, chitosan-SCMC complex, microparticles with different crosslinkers, NSO loaded microparticles, isoniazid loaded microparticles and isoniazid. TGA curves for chitosan-SCMC complex and microparticles were quite similar to each other except that thermal stability was improved by crosslinking. The decomposition temperature of chitosan-SCMC complex was lower than that of chitosan and SCMC. The decreasing decomposition temperature of the complex could be explained as the ionic interaction between COONa and NH_3 groups negatively influenced the strength of the related bond. Similar types of findings were reported in literature[20]. However, the weight loss of complex before decomposition was lower than that of both chitosan and SCMC.

Temperature of decomposition values for crosslinked microparticles 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. NSO and drug release studies also supported the above

observation. Temperature of decomposition values for both NSO and isoniazid loaded crosslinked microparticles were higher than the complex without crosslinking with glutaraldehyde (fig.15).

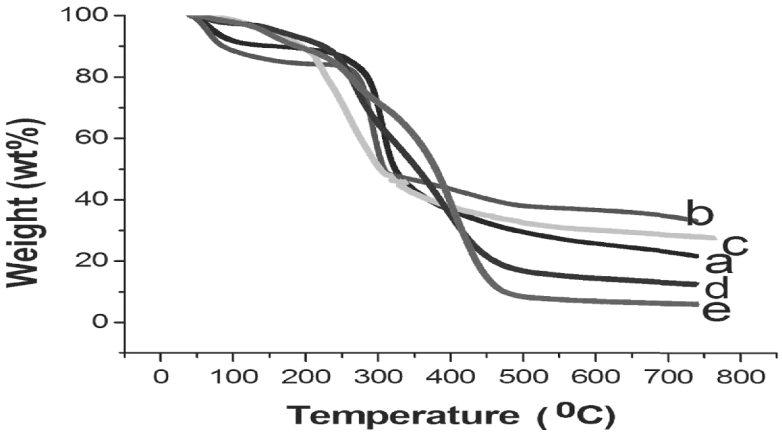

Fig.14: Thermogravimetric curves of (a) chitosan (b) SCMC (c) chitosan-SCMC complex (d) crosslinked microparticles, glutaraldehyde 2.25mmol (e) crosslinked microparticles, glutaraldehyde 6.50 mmol

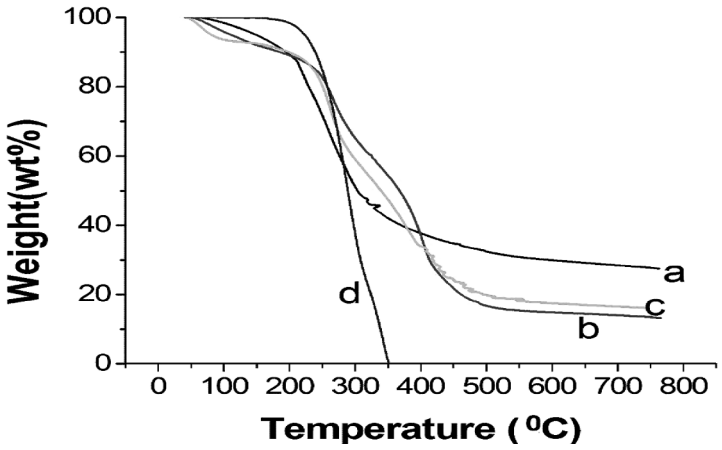

Fig.15: Thermogravimetric curves of (a) chitosan-SCMC complex (b) NSO loaded microparticles (c) isoniazid loaded microparticles (d) isoniazid

4. Conclusion

NSO and isoniazid could be encapsulated efficiently using chitosan-SCMC as complex and and glutaraldehyde as crosslinker. Maximum complexation occurred at 1:2.33 chitosan-SCMC ratio and 2.5-3.5 pH. The

oil encapsulation efficiency was found to increase with the increase in the concentration of NSO, glutaraldehyde and total polymer concentration. Isoniazid loading efficiency of the microparticles increased with the increase in the time of immersion in isoniazid solution. Crosslinking improved thermal stability and also controlled the release of NSO/isoniazid. SEM study revealed that the sizes of the microparticles were decreased as the polymer concentration decreased. Tween 80 further reduced the size of the microparticles. FTIR study confirmed the formation of chitosan-SCMC complex. FTIR study showed no interaction between NSO/isoniazid and chitosan-SCMC complex.

Acknowledgement

The research has been sponsored by the University Grants Commission under the UGC Dr. D. S. Kothari Post Doctoral fellowship Scheme. The author (N. D.) is grateful to UGC for financial assistance.

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