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Research Article

Preparation and Evaluation of Gelatin/Sodium Carboxymethyl Cellulose Polyelectrolyte Complex Microparticles for Controlled Delivery of Isoniazid

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Abstract. The ratio of gelatin to sodium carboxymethyl cellulose (SCMC) at which maximum yield was obtained was optimized. This optimized ratio of gelatin to SCMC along with other parameters was used to prepare microparticles of different sizes. Vegetable oil was used as emulsion medium. Effect of various factors like amount of surfactant, concentration of polymer on the formation, and size of the microparticles was investigated. These microparticles were used as carrier for isoniazid. Among different cross-linkers, glutaraldehyde was found to be the most effective cross-linker at the temperature and pH at which the reaction was carried out. The loading efficiency and release behavior of loaded microparticles were found to be dependent on the amount of cross-linker used, concentration of drug, and time of immersion. Maximum drug loading efficiency was observed at higher immersion time. The release rate of isoniazid was more at higher pH compared to that of at lower pH. The sizes of the microparticles were investigated by scanning electron microscope. In all the cases, the microparticles formed were found spherical in shape except to those at low stirring speed where they were agglomerated. Fourier transform infrared study indicated the successful incorporation of isoniazid into the microparticles. Differential scanning calorimetry study showed a molecular level dispersion of isoniazid in the microparticles. X-ray diffraction study revealed the development of some crystallinity due to the encapsulation of isoniazid.

KEY WORDS: characterization; gelatin; isoniazid; microparticle; sodium carboxy methyl cellulose.

INTRODUCTION

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 (1). 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. Recent implementation of the World Health Organization's strategy (directly observed therapy, short course) has been problematic, and TB remains a major burden in many developing countries. One of the major problems is noncompliance 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 (2). 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.

An important consideration in the treatment of TB is that the etiological agent, *M. tuberculosis*, has the ability to persist intracellularly in the host macrophage for long periods of time. Optimum therapy, therefore, must depend upon the intracellular delivery of antimycobacterial agents for prolonged periods. This becomes even more important when one considers the ability of *M. tuberculosis* to persist in a dormant state, thus giving rise to a large group of infected individuals who carry the organism in a subclinical state without having active disease (3).

Properly devised delivery techniques should theoretically circumvent these problems by positioning effective drugs within host macrophages, thus giving direct access to dormant organisms that presumably would be within macrophages or in the surrounding lymphatic area (4). In the case of a drug that is effective against actively multiplying mycobacteria, this would be advantageous because the drug would continually be available for prolonged periods at the site in the event the organism underwent any multiplication cycle. Microsphere technology has the capability of accomplishing these goals by achieving intracellular delivery of antimycobacterial drugs and allowing programmed controlled release over a prolonged period (5).

Various carrier systems such as liposomes (6,7), polymers (8–10), and microcontainers (11) are used as antitubercular drug carriers. Although the experience with synthetic poly-

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mers is extensive and encouraging (12–14), the recent trend has been to shift towards natural polymers (15). The major advantage of natural polymers includes their low cost and compatibility with the encapsulation of wide range of drugs, with minimal use of organic solvents (16). Furthermore, bioadhesion, stability, safety, and their approval for human use by the US FDA are additional advantages (15,17).

Gelatin is an amphoteric protein and is positively charged below its isoelectric point. Sodium carboxymethyl cellulose (SCMC) is an anionic derivative of cellulose. They are, thus, expected to interact and form polyelectrolyte complex under controlled conditions. The use of gelatin–sodium carboxymethyl cellulose interpenetrating polymer network for controlled delivery of drugs has been cited in the literature (18). The purpose of this study was to prepare and evaluate the polyelectrolyte complex of gelatin A and SCMC as new controlled drug release system for isoniazid. Cross-linking agents are generally employed in order to increase the efficiency of controlled-release system. Varieties of cross-linking agents have been tried. Citric acid did not produce sufficient cross-linking at the temperature (room temperature) at which the reaction was carried out. Genipin, a naturally occurring cross-linker, was found efficient at higher pH (6.0–7.0). Glutaraldehyde was found efficient at the pH (3.5) at which the reactions were carried out. Besides, this process involves 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 (19,20). Another purpose of this study was to investigate the optimal conditions for the formation of microparticles of desired size and hence the dependence of drug encapsulation efficiency and release on the reaction conditions.

MATERIALS AND METHODS

Materials

SCMC (medium viscosity) was purchased from Rankem (India). Gelatin type A was purchased from Sigma-Aldrich Inc. (USA). Glacial acetic acid (E. Merck, India), Tween 80 (E. Merck, India), and glutaraldehyde 25% *w*/*v* (E. Merck, Germany) were used as received. Isoniazid was purchased from Sigma-Aldrich Inc. (USA). Edible-grade refined sunflower oil was purchased from local market. Double-distilled deionized (DDI) water was used throughout the study. Other reagents used were of analytical grade.

Microparticle Preparation

Polyelectrolyte Complexation Conditions

Formation of a polyelectrolyte depends on several parameters such as pH of the polymer solutions, ratio between the polymers, temperature, etc. The optimal conditions for the formation of polyelectrolyte complex of gelatin/SCMC were evaluated by determining the percent yield at various ratios of SCMC to gelatin and at various pH conditions. All the successive experiments were performed at this optimal pH and polymer ratio. The optimum ratio of SCMC to gelatin and pH at which maximum complexation (judged by the percent yield) occurred were 1.0:2.33 and 3.5, respectively.

Preparation Procedure of Microparticles and Microencapsulation

To a beaker containing 150–350 ml of sunflower oil, 15–50 ml of gelatin A solution (2–4% *w*/*v*) was added under stirring condition (200–1,500 rpm approximately) at 60±1°C to form an emulsion. Zero gram to 1 g of the Tween 80, dissolved in 10 ml of water, was added to the beaker to stabilize the emulsion. Fifteen milliliters to 50 ml of SCMC solution of concentration 0.857–1.714% (*w*/*v*) was added to the beaker dropwise to attain complete phase separation. However, the weight ratio of SCMC to gelatin was maintained at 1:2.33 during all the experiments. The pH of the mixture was then brought down to 3.5 by adding 2.5% (*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 down slowly for 30 min to bring down the temperature to 5–10°C by replacing the hot water from water bath with ice cluster. This was done to harden the microparticles. The cross-linking of the polymer microparticles was achieved by slow addition of a certain amount of glutaraldehyde (4.375–17.50 mmol/g of polymer) solution. The temperature of the beaker was then allowed to rise to room temperature, and stirring was continued for another 10–11 h to complete the cross-linking reaction. The microparticles were filtered through 300-mesh nylon cloth, washed with acetone to remove oil, if any, and adhered to the surface of microparticles. This was further washed with distilled water and freeze-dried. The dried microparticles were then dipped in aqueous isoniazid solution (0.5–20%, *w*/*v*) for different times (0.5–48 h), 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.

Calibration Curve of Isoniazid

A calibration curve is required for the determination of release rate of isoniazid from the microparticles. 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 of 0.001 to 0.01 g/100 ml, a prominent peak at 261 nm was noticed. The absorbance values at 261 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of isoniazid was obtained by knowing the absorbance value.

Loading Efficiency

Microparticles were first grounded in a mortar; 0.3 g of accurately weighed grounded materials was transferred with precaution to a volumetric flask containing 100 ml of phosphate buffer solution (pH=7.4, ionic strength 0.01 at 30°C) and kept overnight with continuous stirring to dissolve the isoniazid in the microparticles. The solution was allowed to settle down, filtered, and collected. The isoniazid inside the

microparticles was determined, employing UV spectrophotometer. The loading efficiency (%) was calculated by using the calibration curve and the following formula

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

where

Nomenclature

w_1 amount of isoniazid encapsulated in a known amount of microparticles
w_2 weight of microparticles

Drug Release Studies

Isoniazid release studies from the isoniazid-encapsulated microparticles were carried out with the help of absorbance readings by using UV-visible spectrophotometer (UV-2001 Hitachi); 0.3 g of microparticles was taken into a known volume (100 ml) of dissolution media (pH=1.2 and 7.4). The pH of the medium 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 dissolution medium was returned to the container.

Water Uptake Study

The swelling behavior of SCMC–gelatin microparticles was studied in two systems at pH=1.2 (0.1 N HCl) and pH=7.4 (phosphate buffer). Microparticles (0.3 g) were taken in a pouch made of nylon cloth. The empty pouch was first conditioned by immersing it in either 0.1 N HCl (pH 1.2) or phosphate buffer (pH 7.4) for different time periods (1–24 h). The pouch containing the microparticles was immersed in a similar way in either 0.1 N HCl (pH 1.2) or phosphate buffer (pH 7.4) for the similar time periods. The weights of wet microparticles at a definite time period were determined by deducting the respective conditioned weight of the empty nylon pouch from this.

The water uptake (%) was determined by measuring the change of the weight of the microparticles. The percentage of water uptake for each sample determined at time t was calculated using the following equation.

$$\text{Water uptake}(\%) = [(W_t - W_0)/W_0] \times 100$$

where W_t is the weight of the wet microparticles after allowing to swell for a time (t), and W_0 is the initial weight of the microparticles before swelling. The experiments were performed in triplicate and represented as a mean value.

Scanning Electron Microscopy Study

The samples were deposited on a brass holder and sputtered with platinum. Sizes of the microparticles were studied at room temperature using scanning electron microscope (model JEOL, JSM-6390) at an accelerated voltage of 5 kV.

Fourier Transform Infrared Study

Fourier transform infrared (FTIR) spectra were recorded in the range of 4,000–400 cm^{-1} using KBr pellet in a Nicholet (model Impact-410) spectrophotometer. Gelatin A, SCMC, polyelectrolyte complex of gelatin A and SCMC, isoniazid, and isoniazid-containing microparticles were each separately finely grounded with KBr and thus kept ready for taking spectra.

X-ray Diffraction Study

X-ray diffractograms of gelatin A–SCMC microparticles, isoniazid, and microparticles with isoniazid encapsulation were recorded on an X-ray diffractometer (Model MiniFlex, Rigaku Corporation, Japan). The samples were scanned between $2\theta=3°$ and 50° at a scan rate of 4°/min.

Thermal Property Study

Thermal properties of SCMC–gelatin microparticles, isoniazid, and isoniazid-loaded microparticles were evaluated by employing differential scanning calorimeter (DSC).The samples were hermetically sealed in a pan, and a study was done in a differential scanning calorimeter (model DSC-60, Shimadzu) at a heating rate of 10°C/min up to 450°C. All studies were done under nitrogen atmosphere.

RESULTS AND DISCUSSION

Effect of Variation of Amount of Surfactant and Polymer Concentration on Size of the Microparticles

The formation and particle size of each microparticle depend on the size of the dispersed droplet, which is determined by the surfactant (Tween 80) used and the emulsifying conditions. For the system of SCMC–gelatin, surfactant Tween-80 had an important role in stabilizing the microparticles formed in sunflower oil. A matrix gel-like product was formed if the surfactant was not added. But different sizes of microparticles were formed on addition of varying amounts of surfactant. SEM photographs of the microparticles are shown in Fig. 1. With the increase of amount of Tween-80 from 0.22 g to 1.16 g/g of polymer, the sizes of the microparticles decreased as shown in Fig. 1a–c. 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. Landfester also reported the same type of phenomena that, with the increase of surfactant, the size of the droplets in miniemulsions decreased (21).

Again, with increase of the amount of polymer from 0.857 to 1.714 g, an increase in the size of the microparticles was observed (Fig. 1c–e). 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

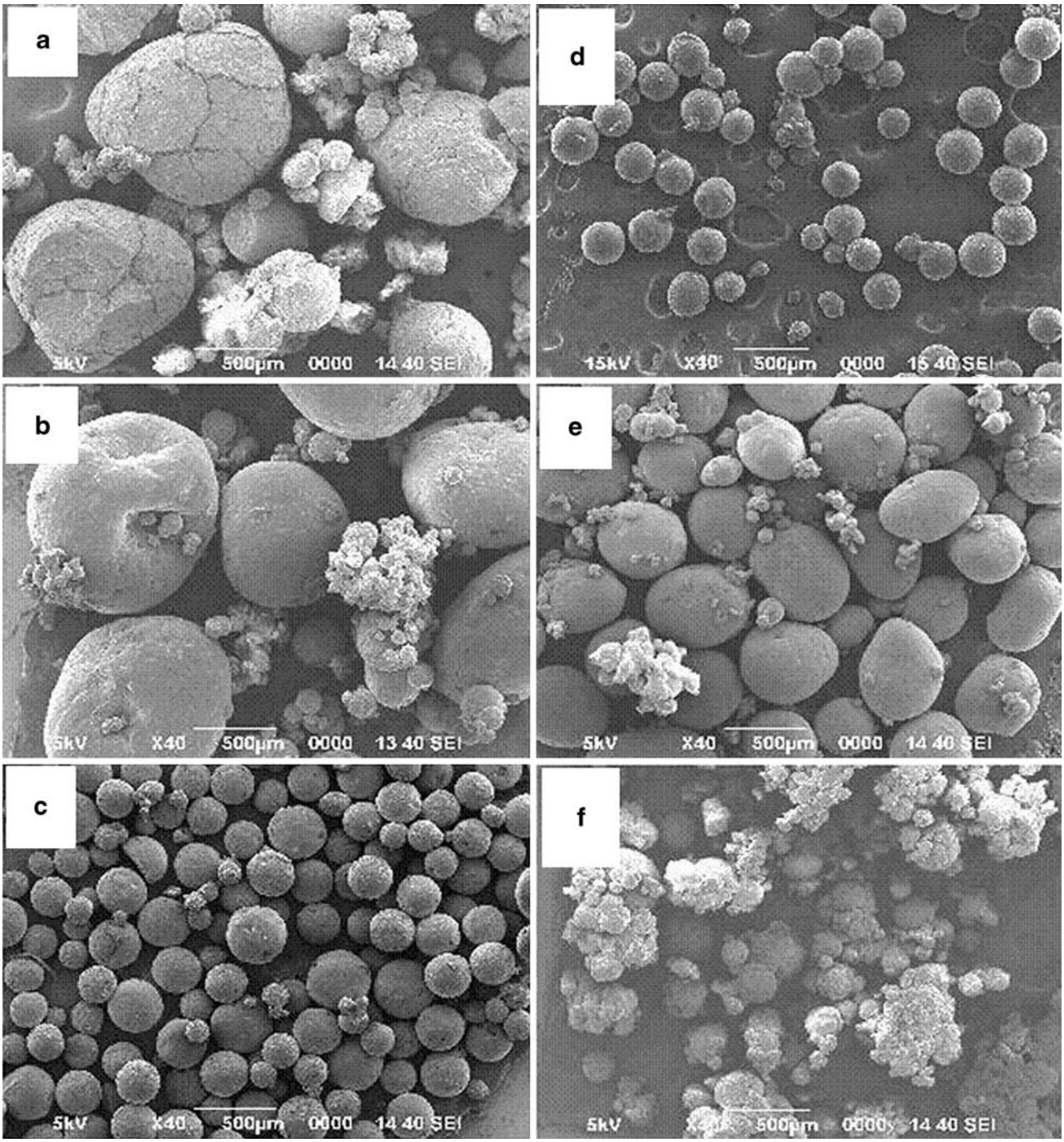

Fig. 1. Scanning electron micrographs of microparticles prepared by using **a** Tween 80=0.22 g/g of polymer, total polymer=0.857.0 g; **b** Tween 80=0.46 g/g of polymer, total polymer=0.8570 g; **c** Tween 80=1.16 g/g of polymer, total polymer=0.857 g; **d** Tween 80=1.16 g/g of polymer, total polymer=1.428 g; **e** Tween 80=1.16 g/g of polymer, total polymer=1.714 g; **f** Tween 80=1.16 g/g of polymer, total polymer=0.857 (low stirrer speed)

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 the presence of higher amount of polymer and as a result larger microparticles were formed (Fig. 1c–e).

Stirring speed also affected the nature and size of the microparticles. At low stirrer speed (200 rpm approximately), the agglomeration of particles was more (Fig. 1f) compared to those of particles produced at higher stirrer speed (1,500 rpm approximately; Fig. 1d). Improper mixing of polymers at low stirrer speed might be responsible for the observed agglomeration. Zhuo *et al.* reported similar observations during the study of the particle size of polyurea microcapsules by interfacial polymerization of polyisocyanates (22).

All the microparticles were spherical in shape except the particles produced at low stirrer speed. The microparticles

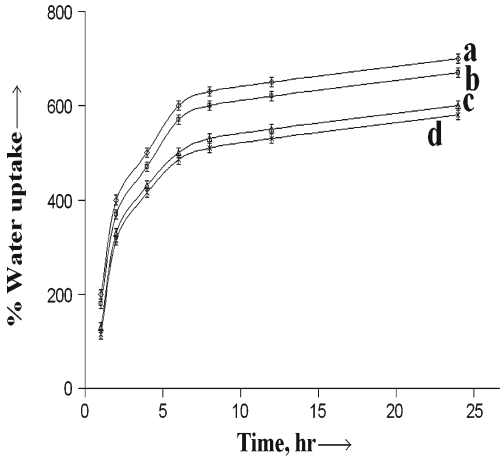

Fig. 2. Effect of variation cross-linker amount and pH on water uptake (*a*: polymer=2.857 g, cross-linker=4.375 mmol/g of polymer, pH=7.4; *b*: polymer=2.857 g, cross-linker=4.375 mmol/g of polymer, pH=1.2; *c*: polymer=2.857 g, cross-linker=17.50 mmol/g of polymer, pH=7.4; *d*: polymer=2.857 g, cross-linker=17.50 mmol/g of polymer, pH=1.2)

arising from low stirrer speed had no definite structure probably due to agglomeration. Again, some roughness appeared on the surfaces of all the microparticles (Fig. 1a–d). The surface of the microparticles prepared at higher polymer and surfactant concentration (Fig. 1e) showed least roughness compared to those of other particles.

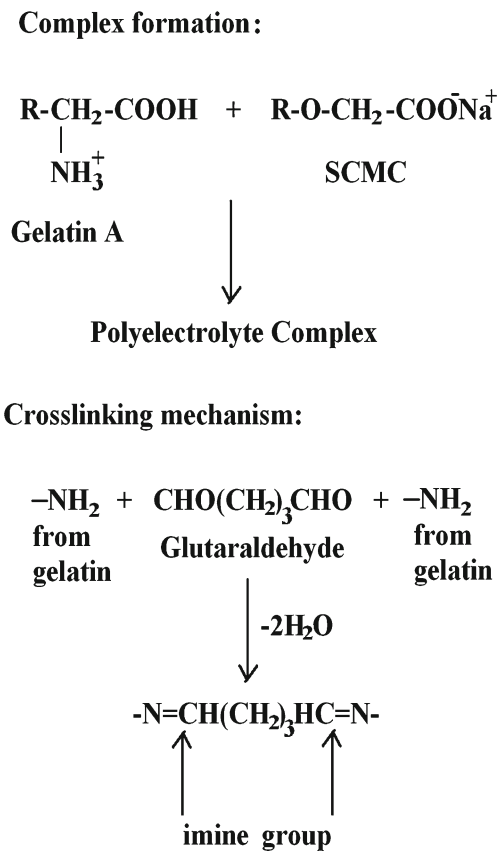

Fig. 3. Probable reaction scheme for interaction between SCMC and gelatin and glutaraldehyde with SCMC–gelatin complex

Water Uptake Study

The water uptake of microparticles was plotted against time of immersion in water (Fig. 2). The water uptake increased rapidly up to 2 h then slowly until 5 h as shown and finally almost leveled off. Microparticles with higher cross-linking showed lesser water uptake than the microparticles with low cross-linking. This was due to the formation of more compact wall (23) caused by cross-linking. Probable reaction scheme for complexation between the two polymers and cross-linking with glutaraldehyde is shown in Fig. 3. Again, water uptake was more at higher pH compared to lower pH. Microparticles formed by the complexation between gelatin A and SCMC became more stable probably at lower pH. At higher pH, the tendency to decomplex between gelatin and SCMC might be responsible for the higher water uptake capacity. Similar findings were reported by Liu *et al.* (24) during the study of the swelling behavior of gelatin–DNA semi-interpenetrating polymer network at different pH.

Table I. Effect of Variation of Isoniazid Concentration and Immersion Time on Loading Efficiency

Concentration of glutaraldehyde (mmol/g of polymer)	Concentration of isoniazid (g/100 ml)	Time of immersion (h)	Loading efficiency (%)
4.375	0.5	1.5	0.33±0.01
	1.0		0.99±0.12
	3.0		14.33±0.11
	5.0		28.66±0.17
	7.0		44.0±0.21
	10.0		51.0±0.43
	20.0		58.11±0.55
10.50	0.5	1.5	0.33±0.01
	1.0		0.66±0.11
	3.0		14.0±0.16
	5.0		23.11±0.18
	7.0		40.21±0.35
	10.0		46.33±0.39
	20.0		50.00±0.52
17.50	0.5	1.5	0.33±0.01
	1.0		0.66±0.13
	3.0		14.0±0.32
	5.0		22.0±0.47
	7.0		38.79±0.58
	10.0		42.11±0.59
	20.0		46.23±0.63
17.50	5.0	0.5	19.0±0.25
		1.0	28.0±0.31
		2.0	35.0±0.42
		4.0	36.0±0.41
		6.0	36.45±0.35
		8.0	37.12±0.32
		17.0	46.0±0.46
		28.0	58.0±0.38
		48.0	58.50±0.31
4.375	20.0	48.0	63.2±0.53
10.50	20.0	48.0	62.0±0.61
17.50	20.0	48.0	60.7±0.50

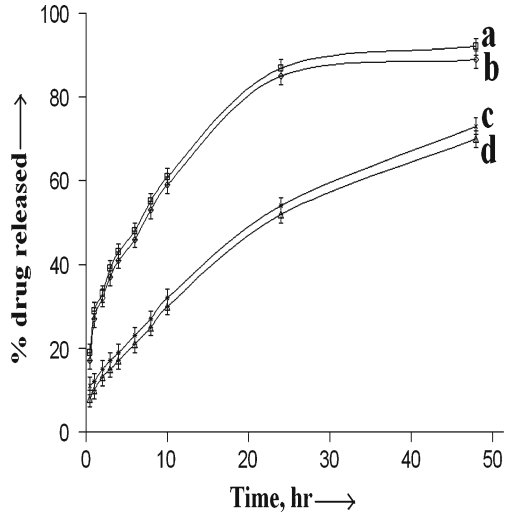

Fig. 4. Effect of variation of cross-linker concentration and pH on release profile (*a*: polymer=2.857 g, cross-linker=4.375 mmol/g of polymer, pH=7.4; *b*: polymer =2.857 g, cross-linker=4.375 mmol/g of polymer, pH=1.2; *c*: polymer=2.857 g, cross-linker=17.50 mmol/g of polymer, pH=7.4; *d*: polymer=2.857 g, cross-linker=17.50 mmol/g of polymer, pH=1.2)

Effect of Variation Drug Concentration and Immersion Time on Loading Efficiency

The effect of variation of concentration of isoniazid and immersion time of microparticles on loading efficiency is shown in Table I. At a fixed immersion time, the loading efficiency was found to increase with the increase in the concentration of isoniazid. An increase in loading efficiency was also observed as immersion time increased. Again, the higher the amount of cross-linker in the microparticles, the lower was the loading efficiency. The

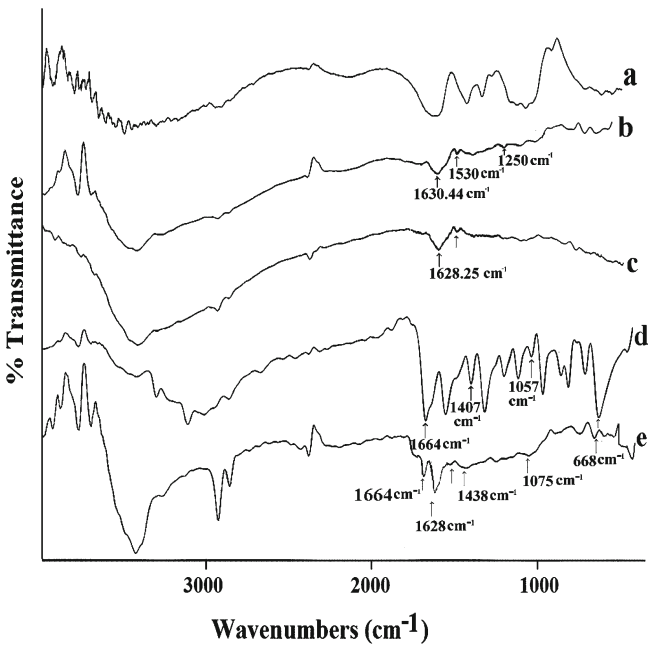

Fig. 5. FTIR spectra of *a* carrageenan, *b* SCMC, *c* gelatin–SCMC complex, *d* isoniazid, *e* isoniazid-loaded microcapsules

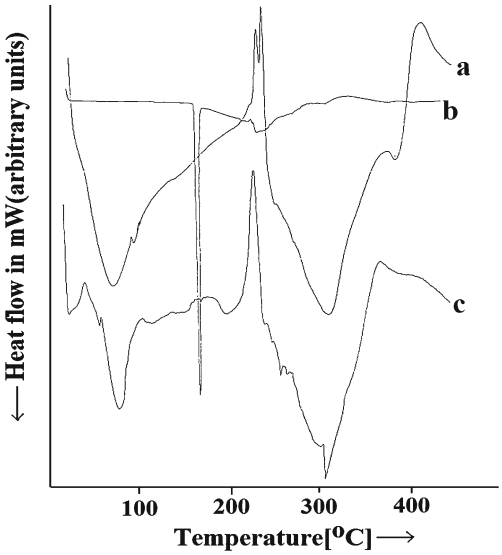

Fig. 6. DSC thermograms of *a* SCMC–gelatin complex, *b* isoniazid, *c* isoniazid-loaded microcapsules

increase in loading efficiency was due to more diffusion of isoniazid into the microparticles. The decrease in loading efficiency might be attributed to the formation of more compact wall due to cross-linking that led to a decrease in diffusion rate of isoniazid.

Again, at a fixed isoniazid concentration, the loading efficiency increased with time of immersion up to a certain time and after that it remained constant. But high and low cross-linked microparticles showed more or less similar loading efficiency when immersed in similar concentration of isoniazid solution for a longer period. Longer immersion time allowed the microparticles to become saturated with isoniazid solution.

Effect of Variation of Cross-linker on Release Rate of Isoniazid

The effect of the variation of cross-linker concentration (4.375–17.50 mmol/g of polymer) on release rate at pH 1.2 and 7.4 is shown in Fig. 4. Microparticles having approx-

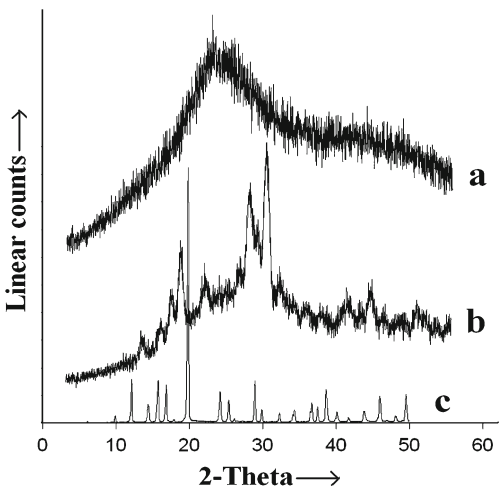

Fig. 7. X-ray diffractograms of *a* microspheres without isoniazid, *b* isoniazid, *c* isoniazid-loaded microspheres

imately similar loading were chosen for the study of the release rate at different pH. The release rate of isoniazid was found to decrease with the increase in the amount of cross-linker in the microparticles. In all the cases, the release was fast initially, reaching maximum, and leveled off finally. The compact microparticle wall was responsible for the decrease in release rate as explained earlier.

Further, the percentage of isoniazid release at lower pH (pH=1.2) was less compared to that of at higher pH (pH= 7.4). The lower and higher release rate in lower and higher pH, respectively, might be explained by considering the tendency of complexation and decomplexation between gelatin A and SCMC as discussed earlier.

Fourier Transform Infrared Study

The spectra of SCMC (curve a), gelatin A (curve b), SCMC–gelatin complex (curve c), isoniazid (curve d), isoniazid-loaded cross-linked SCMC–gelatin microparticles (curve e) are shown in Fig. 5. The spectrum of SCMC showed absorption bands at 3,364, 2,942, 1,627, 1,422, 1,063 cm^{-1} which were due to O–H stretching vibration, CH_3 symmetric + CH_2 asymmetric vibration, C–O stretching band for cellulose, CH_3 + CH_2 bending vibration, and strong C–O stretching band for ethers. The notable absorption bands for gelatin A appeared at 3,421 cm^{-1} (NH stretching), 1,630.44 cm^{-1} (amide I, CO and CN stretching),1,530 cm^{-1} (amide II), and 1,250 cm^{-1} (amide III). Among the absorption bands, the amide I band between 1,600 and 1,700 cm^{-1} is the most important peak for IR analysis of the secondary structure of protein-like gelatin (25). In the complex of gelatin and SCMC, a slight shift of the peak of amide I from 1,630.44 to 1,628.25 cm^{-1} was observed. This indicated that the negatively charged carboxy methyl groups might associate with positively charged gelatin. A similar type of observation was reported by Pranoto *et al.* (26) while studying the interaction between carrageenan and gelatin. The probable interaction between SCMC and gelatin is shown in Fig. 3. In the spectrum (shown as curve d) of isoniazid, the carbonyl absorption (amide I band) appeared at 1,664 cm^{-1}. The amide II band that occurred at 1,555.90 cm^{-1} was due to N–H bending of the secondary amide group. Moreover, in the spectrum of isoniazid, multiple bands appeared between 1,400 and 668 cm^{-1}. Some of the characteristic bands of isoniazid appeared in the isoniazid-loaded microparticles (curve e), suggesting the successful loading of isoniazid in the microparticles. A similar type of IR spectral pattern for isoniazid and isoniazid-containing capsules was reported by Kim *et al*. (12).

Thermal Property Study

DSC thermograms of SCMC–gelatin complex (curve a), isoniazid (curve b), and isoniazid-loaded microparticles (curve c) are shown in Fig. 6. The endotherm appeared in all the thermograms except isoniazid at around 100°C, which was due to removal of moisture. The thermograms of isoniazid showed an endothermic peak due to melting at around 190°C. There was no characteristic peak of isoniazid in the thermogram of isoniazid-loaded microparticles. These results indicated that isoniazid was dispersed in the microparticles. A similar observation was reported by Patil *et al.* during DSC analysis of carvedilol drug encapsulated within alginate microspheres (27).

X-ray Diffraction Study

X-ray diffractograms of gelatin–SCMC microparticles (curve a), isoniazid-loaded micro particles (curve b), and isoniazid (curve c) are shown in Fig. 7. Isoniazid showed multiple sharp peaks at 2θ, varying from 12° to 50°, which were due to the crystalline nature of isoniazid. Appearance of some of these peaks in the diffractograms of isoniazid-loaded microparticles indicated development of some crystallinity due to the encapsulation of isoniazid.

CONCLUSIONS

The optimum conditions for maximum complexation between carrageenan and gelatin occurred at SCMC to gelatin ratio of 1.0:2.33 and pH 3.5. Microparticles of various sizes were prepared by varying surfactant and polymer concentration. Isoniazid concentration governed the absorption of isoniazid into the microparticles. Higher pH medium facilitated the release of isoniazid more compared to lower pH. FTIR study indicated the loading of isoniazid into the microparticles. DSC studies showed that isoniazid was dispersed in the microparticles. X-ray study indicated the development of some crystallinity due to encapsulation of isoniazid.

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