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Cross-linked Chitosan Microspheres: Preparation and Evaluation as a Matrix for the Controlled Release of Pharmaceuticals

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Abstract—Chitosan microspheres having good spherical geometry and a smooth surface were prepared by the glutaraldehyde cross-linking of an aqueous acetic acid dispersion of chitosan in paraffin oil using dioctyl sulphosuccinate as the stabilizing agent. Microspheres having different degrees of swelling were made by varying the cross-linking density. Microspheres were prepared by incorporating theophylline, aspirin or griseofulvin. Drug incorporation efficiencies exceeding 80% could be achieved for these drugs. In-vitro release studies of these drugs were carried out in simulated gastric and intestinal fluids at 37°C. It was observed that the drug release rates were influenced by the cross-linking density, particle size and initial drug loading in the microspheres.

There has been considerable interest in recent years in developing controlled or sustained drug delivery systems using polymeric microspheres (Davis et al 1984). Chitosan is a hydrolysed derivative of chitin, a biopolymer widely distributed in nature. Chitosan has attracted attention as a matrix for controlled release since it possesses reactive functionalities, is easily degraded by enzymes and the degradation products are non-toxic (Muzzarelli 1977). The characteristic insolubility of chitosan in water, as well as in many common organic solvents, limits its use as a drug carrier. Nagai et al (1984) prepared compressed tablets of drug and chitosan and followed the drug release behaviour in-vitro. Chitosan is reported to find application as a drug in the treatment of hyperbilirubinaemia and hypercholesterolaemia (Nagyvary 1982; Furda 1980). Chitin and chitosan have been reported to possess the characteristic property of selectively concentrating in tumour cells. Chitosan carrying 5-fluorouracil has been prepared and evaluated for its antitumour activity (Ouchi et al 1989). Thus, chitosan appears to be a promising matrix for the controlled release of pharmaceutical agents. In view of the biodegradable and non-toxic nature of chitosan, chitosan microspheres may also find application as particulate emboli in endovascular embolization (Benoit & Puisieux 1986). In this paper we report a procedure for the preparation of chitosan microspheres of good spherical geometry and strength. A preliminary evaluation of the release characteristics of the oral drugs theophylline, aspirin and griseofulvin incorporated into chitosan microspheres in simulated gastric and intestinal fluids is also reported.

Materials and Methods

Materials

Chitosan (Purified, Viscosity Grade 50) obtained from Central Institute of Fisheries and Technology, Cochin, India, was used without further purification. Theophylline, dioctyl sulphosuccinate (DOS), and glutaraldehyde (25% aqueous solution) purchased from Sigma Chemical Company, USA, were used directly. Griseofulvin was obtained from IDPL, Baroda, India, and was used after recrystallization from dichloroethane. Aspirin was synthesized and recrystallized twice before use (Vogel 1978). Liquid paraffin of two different viscosities (90 cP at 30°C, heavy) and (18 cP at 30°C, light) were from SD Fine Chemicals, Bombay, India. All other solvents and reagents were of analytical or equivalent grade.

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Methods

A 4% solution of chitosan was prepared in 5% aqueous acetic acid. This solution (26 g) was dispersed in 150 mL of liquid paraffin (1:1 mixture of light and heavy) containing 0·15 g DOS in a 250 mL beaker. The dispersion was stirred using a stainless steel half-moon paddle stirrer at 500 rev min^{-1} ($\pm$10%) for 2 min, and 4 mL of glutaraldehyde-saturated toluene (Longo et al 1982) was added and the stirring continued at room temperature (27°C). More toluene or aqueous glutaraldehyde solution was added at 30 min intervals for preparing microspheres of different cross-linking densities and the reaction was continued for a total of 3 h. Unless specified, a typical cross-linking reaction was carried out by adding 4 mL of glutaraldehyde-saturated toluene initially, followed by another 4 mL of the same at the end of 30 min and 5 mL of 25% aqueous glutaraldehyde at the end of 1 h. After the reaction, the beads were filtered off, washed several times with hexane, methanol and finally with water. The beads were then dried in an air oven at 50°C. Drug-loaded microspheres were also prepared in a similar fashion by mixing the required amount of drug with the chitosan solution and cross-linking the matrix as before. Washing with methanol was, however, avoided in the case of drug-loaded spheres. Spheres were washed with plenty of ice-cold water to remove the acetic acid and glutaraldehyde. Microspheres were fractionated by sieving them through standard test sieves (Filterwel, Bombay, India).

The swelling ability of the microspheres in various physiological media was determined by swelling them to their equilibrium in various fluids and estimating their equilibrium fluid content (Thanoo & Jayakrishnan 1990).

In-vitro release profiles of the drugs from the microspheres were examined in simulated gastric and intestinal fluids without enzymes (US Pharmacopoea 1985). One hundred mg of the drug-loaded microspheres in the size range 425–600 μm was added to 500 mL of the dissolution medium in a 1000 mL Erlenmeyer flask and shaken in a bath incubator shaker at 37°C. Samples (0·5 mL) were withdrawn at intervals and assayed spectrophotometrically in a UV-Vis spectrophotometer (Shimadzu, UV 240, Japan) at 274 for theophylline, 277 for aspirin and 291 nm for griseofulvin. To maintain a constant volume of the dissolution medium, an amount equal to the volume withdrawn was immediately added after each withdrawal. The total drug present in the microspheres was estimated by extracting the microspheres with methanol for 24 h. The drug incorporation efficiency was then calculated from the ratio of actual and theoretical drug content.

Scanning electron microscopy was performed using a Jeol instrument (JSM-35C) after coating the microspheres with palladium/gold.

Results and Discussion

Chitosan, which is insoluble in water, can be solubilized in dilute acetic acid due to the formation of the acetate salt (Austen 1984; Austen & Sennet 1986). The viscosity of a 4% solution in 5% acetic acid was found to be 850 cP at 30°C and was the optimum for dispersing into droplets even with high (>70%) drug loadings. A more concentrated solution was found to be too viscous for obtaining good dispersibility in liquid paraffin. Glutaraldehyde cross-linking of chitosan is an instantaneous reaction. When aqueous glutaraldehyde was added to the dispersion of chitosan in paraffin oil, instantaneous cross-linking occurred and the product obtained did not exhibit good spherical geometry and surface smoothness. Therefore slow and uniform cross-linking of the droplets particularly on the surface was felt desirable to generate spheres of good sphericity. Hence glutaraldehyde-saturated toluene was chosen instead of an aqueous solution of glutaraldehude to induce the cross-linking. Toluene saturated with glutaraldehyde, because of its solubility in the oil medium would be uniformly available for cross-linking the surface of the droplets. The surface hardening of the droplets by cross-linking thus fix the shape and surface morphology of the microspheres. Further cross-linking and hardening of the droplets could be effected by the addition of more glutaraldehyde once the surface hardening had been achieved.

The swelling of chitosan beads is influenced by the extent of cross-linking induced. Table 1 shows the effect of cross-linking on the equilibrium water content (EWC%) of chitosan beads. Beads having an EWC of more than 90% could be prepared by controlling the cross-linking density. The swelling of the beads in various physiological media as determined by the equilibrium fluid content (EFC%) is shown in Table 2. The degree of swelling was found to be the same in all the fluids tested indicating very little effect of the ionic strength or pH of the medium.

Incorporation of drugs in the microspheres altered the surface morphology of the microspheres to a significant extent. Fig. 1 shows the scanning electron micrograph of chitosan microspheres with and without the drug. Incorporation of a significant quantity (>50%) of griseofulvin

Table 1. Equilibrium water content of chitosan microspheres having different cross-linking densities.

Recipe	Glutaraldehyde-saturated toluene used for cross-linking (mL)	Equilibrium water content* (%)
a	1	93·7±0·5
b	2	82·2±0·6
c	2+2 at 30 min	72·8±0·5
d	2+2 at 30 min +5 aqueous solution (25%)	52·3±0·2

* Average of three determinations ± s.d.

Table 2. The swelling of cross-linked chitosan microspheres[a] in various fluids at 30°C.

Fluid	Equilibrium fluid content (%)[b]
Water	52·3±0·2
Calcium chloride (1·3%)	56·2±0·4
Sodium chloride (0·9%)	54·6±0·7
Glucose (5·51%)	56·5±0·9
Urea (1·63%)	54·8±0·7
Phosphate buffer (0·5 M, pH 7·4)	52·1±0·8

[a] Microspheres cross-linked using recipe d of Table 1. [b] Average of three determinations ± s.d.

Table 3. Incorporation efficiency of theophylline, aspirin and griseofulvin in chitosan microspheres.

Drug	Theoretical content (%)	Actual content (%)	Incorporation efficiency (%)
Theophylline	22	14	63·6
	41	34	82·9
	63	52	82·5
	76	61	80·2
Griseofulvin	22	9	40·9
	41	31	75·6
	63	52	82·5
	76	73	96·1
Aspirin	22	14	63·6
	63	51	80·9
	76	68	89·5

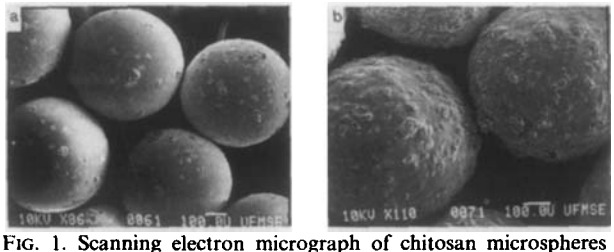

FIG. 1. Scanning electron micrograph of chitosan microspheres without drug (a) and with 52% loading of griseofulvin (b).

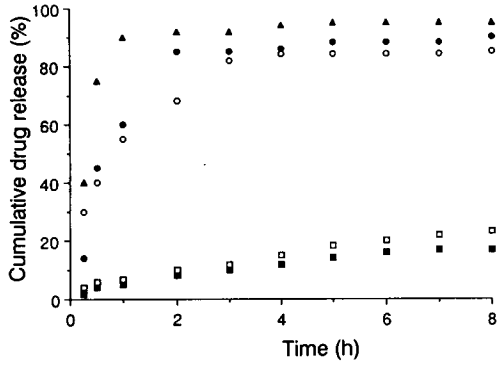

FIG. 2. The release profile of theophylline, aspirin and griseofulvin from cross-linked chitosan microspheres (425–600 μm) in gastric and intestinal fluid at 37°C. Griseofulvin in intestinal (□) and gastric (■) fluid, aspirin in intestinal (●) and gastric (○) fluid, theophylline in intestinal fluid (▲). Values for theophylline in gastric fluid were essentially the same as those in intestinal fluid.

imparts a high degree of surface roughness to the spheres; results were similar for theophylline and aspirin. The drug incorporation efficiencies of the three drugs tested at various initial drug loadings in the microspheres are given in Table 3. The drug loading efficiencies were found to be good with all the three drugs. Both griseofulvin and aspirin showed better loading efficiency compared with the more soluble drug theophylline.

The release profiles of the three drugs in gastric and intestinal fluids are shown in Fig. 2. In the case of theophylline, the release was very fast in both the fluids and almost 95% of the drug was released within 1 h. The release of griseofulvin which is the least soluble drug was very slow in both the fluids; after 8 h, only about 20% of the drug was released. For theophylline and griseofulvin, the release profile was almost the same in both fluids while aspirin showed a slightly faster rate of release in intestinal fluid as compared with gastric fluid probably because acetyl salicylic acid is ionized and solubilized to a higher extent in the slightly alkaline (pH 7·5) intestinal fluid.

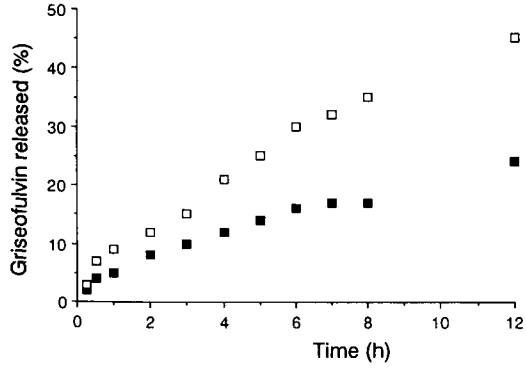

FIG. 3. The release profile of griseofulvin from chitosan microspheres (425–600 μm) having two different cross-linking densities, in intestinal fluid at 37°C. (□) Cross-linked by adding 1 mL of saturated toluene solution and (■) 4 mL of saturated toluene solution initially, followed by 4 mL at the end of 30 min and 5 mL of 25% aqueous solution at the end of 1 h as in a routine preparation. Initial drug loading was 52%.

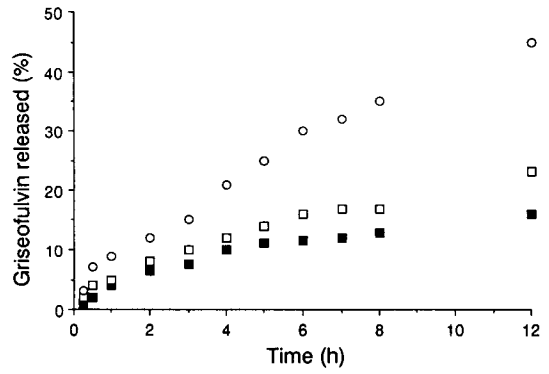

FIG. 4. The release profile of griseofulvin from cross-linked chitosan microspheres (425–600 μm) in intestinal fluid at 37°C. (○) 9%, (□) 52% and (■) 73% griseofulvin loading.

The cross-linking density of the microspheres influenced the release profiles of the drugs. For example, the release rate of griseofulvin from chitosan microspheres cross-linked to two different extents is shown in Fig. 3. The least cross-linked microspheres released the drug at a faster rate while the highly cross-linked microspheres released the drug at a slower rate. Increased cross-linking impeded the swelling of the beads in the dissolution medium as well as diffusion of the drugs, which resulted in lower release rate.

In the case of a water soluble drug such as theophylline, the initial drug loading was not found to influence the rate of drug release to any significant extent (data not given). However, for the less soluble drug, griseofulvin, the rate of release was significantly influenced by the initial loading in the microspheres. A 2- to 3-fold (approx.) increase in the release rate was observed when the drug loading was reduced from 76 to 22% (Fig. 4). It is possible that a higher proportion of the more hydrophobic drug inside the spheres hindered penetration of the dissolution medium into the matrix thereby reducing the solubility of the drug and hence the release rate.

Smaller beads, because of the larger area of contact with the dissolution medium favour more rapid dissolution of the drug in comparison with larger beads. The release rate of griseofulvin could be increased several fold if the particle size was reduced (Fig. 5). Microspheres having 52% drug loading

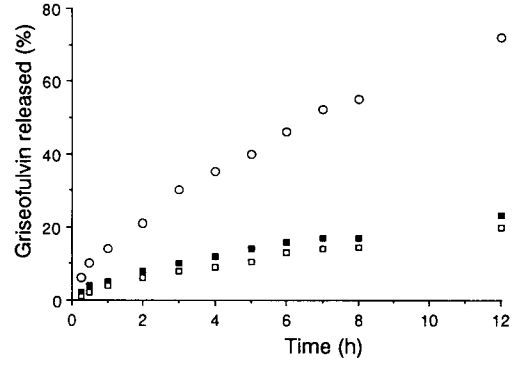

FIG. 5. Effect of bead size on the release profile of griseofulvin from cross-linked chitosan microspheres (drug loading 52%) in intestinal fluid at 37°C. (○ 150–180 μm, ■ 425–600 μm, □ 1000–1200 μm.)

released only 12% of the drug from particles of 1000–1200 μm size whereas more than 50% of the drug was released from particles of 150–180 μm in 8 h.

It appears that chitosan could be used as a suitable matrix as microspheres for the controlled release of pharmaceutical agents. As the release profiles of the drugs are determined by the cross-linking density of the microspheres, their size, and the initial drug content, it offers an opportunity to manipulate these parameters to obtain a nearly zero order release from the matrix. In view of the biodegradable nature of the matrix, chitosan microspheres, provided they can be prepared with particle sizes of < 10 μm, may also prove to be useful for intravenous administration of chemotherapeutic agents as well as in chemoembolization.

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