ELSEVIER

International Journal of Pharmaceutics 249 (2002) 7–21

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Development and in vitro evaluation of novel floating chitosan microcapsules for oral use: comparison with non-floating chitosan microspheres

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Received 12 April 2002; received in revised form 27 June 2002; accepted 15 July 2002

Abstract

Floating (F) microcapsules containing melatonin (MT) were prepared by the ionic interaction of chitosan and a negatively charged surfactant, sodium dioctyl sulfosuccinate (DOS). The DOS/chitosan complex formation was confirmed employing infrared spectroscopy, differential scanning calorimetry (DSC), solubility and X-ray diffraction analysis. The characteristics of the F microcapsules generated compared with the conventional non-floating (NF) microspheres manufactured from chitosan and sodium tripolyphosphate (TPP) were also investigated. The effect of various factors (crosslinking time, DOS and chitosan concentrations, as well as drug/polymer ratio) on microcapsule properties were evaluated. The use of DOS solution in coagulation of chitosan produced well-formed microcapsules with round hollow core and 31.2–59.74% incorporation efficiencies. Chitosan concentration and drug/polymer ratio had a remarkable effect on drug entrapment in DOS/chitosan microcapsules. The dissolution profiles of most of microcapsules showed near zero order kinetics in simulated gastric fluid (S.G.F: pH 1.2). Moreover, release of the drug from these microcapsules was greatly retarded with release lasting for several hours ($t_{50\%}$ (S.G.F.): 1.75–6.7 h, depending on processing factors), compared with NF microspheres where drug release was almost instant. Most of the hollow microcapsules developed tended to float over simulated biofluids for more than 12 h. Swelling studies conducted on various drug-free formulations, clearly indicated that DOS/chitosan microcapsules showed less swelling and no dissolution in S.G.F. for more than 3 days, whereas, TPP/chitosan microspheres were markedly swollen and lost their integrity in S.G.F. within 5 h. Therefore, data obtained suggest that the F hollow microcapsules produced would be an interesting gastroretentive controlled-release delivery system for drugs.

Keywords: Floating chitosan microcapsules; Non-floating chitosan microspheres; Zero order release; Melatonin

1. Introduction

A problem frequently encountered with conventional controlled release dosage forms is the inability to increase their residence time in the stomach and proximal portion of the small intes-

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00396-4

tine. Retention of drug delivery systems in the stomach prolongs overall gastrointestinal transit time, thereby, resulting in improved oral bioavailability of the basic drugs that have poor solubility in higher pH, as well as, drugs susceptible to circadian variations (Menon et al., 1994; Whitehead et al., 1998; Fell, 1999; Baumgartner et al., 2000).

Various approaches have been proposed to retain the dosage form in the stomach. These methods include bioadhesive systems (Deshpande et al., 1996; Santus et al., 1997), swelling and expanding systems (Deshpande et al., 1996, 1997) and floating (F) systems (Menon et al., 1994; Whitehead et al., 1998). In fact, the buoyant dosage unit enhances gastric residence time (GRT) without affecting the intrinsic rate of emptying (Stithit et al., 1998). Unfortunately, F devices administered in a single-unit form (such as hydrodynamically balanced systems (HBS)) are unreliable in prolonging the GRT owing to their 'all-or-nothing' emptying process and, thus, they may cause high variability in bioavailability and local irritation due to a large amount of drug delivered at a particular site of the gastrointestinal tract (GIT) (Whitehead et al., 1998; Lee et al., 1999). In contrast, multiple-unit particulate dosage forms (e.g. microspheres) have the advantages that they pass uniformly through the GIT to avoid the vagaries of gastric emptying and provide an adjustable release, thereby, reducing the intersubject variability in absorption and risk of local irritation (Kawashima et al., 1992; Stithit et al., 1998). Recently, hollow microspheres with a lower density than that of the GI fluids were adopted (Thanoo et al., 1993; Soppimath et al., 2001). The F microspheres were prepared by solvent evaporation techniques using different polymer solution systems such as polycarbonate/dichloromethane (Thanoo et al., 1993), cellulose acetate butyrate/Eudragit RL100 mixture in acetone (Stithit et al., 1998) and Eudragit S100/isopropanol (Lee et al., 1999).

Chitosan is a biocompatible and biodegradable polysaccharide soluble only in aqueous media of low pH and showing extremely low toxicity. It forms gel beads with multivalent counter-ions such as tripolyphosphate (TPP) via ionotropic gelation (Kawashima et al., 1985; Shiraishi et al., 1993; Shu and Zhu, 2000). Great attention has been focused on chitosan as a matrix for the controlled release of active agents, but the formed microspheres showed a limited strength and non-floating (NF) properties in simulated gastric fluid (S.G.F.) (pH 1.2) (Kas, 1997).

Limited investigations have been conducted on the preparation of F microparticulate systems based on chitosan (Miyazaki et al., 1988; Inouye et al., 1989; Lin and Lin, 1992). Recently, He et al. (1999) prepared F cross-linked chitosan microspheres using a novel W/O/W emulsion-spray drying method. Although, this method produced smaller microspheres with a sustained drug release pattern, it is tedious and the use of glutaraldehyde, as well as, chlorinated solvents is undesirable because of their toxicity (Martindale, 1999).

Melatonin (MT: *N*-acetyl-5-methoxytryptamine), a neurohormone secreted by the pineal gland in a circadian fashion, was selected as a model drug. It has a very short half-life of 45 min, and when administered orally in conventional formulations, shows low and variable bioavailability, presumably due to extensive first-pass metabolism (Waldhauser et al., 1984). In addition, the usual nocturnal secretion pattern of MT over 8 h, prompted development of various controlled release dosage forms for treating various circadian rhythm disorders (Lee et al., 1995; Lee and Min, 1996). Therefore, the present work describes F controlled-release MT-loaded chitosan microcapsules with more stable membranes in S.G.F. The microcapsules were prepared, for the first time, via ionic crosslinking of chitosan with sodium dioctyl sulfosuccinate (DOS). The effects of different formulation parameters on the characteristics of chitosan microparticles were studied. The properties of the F microcapsules produced were also compared with those of NF microspheres manufactured from chitosan and TPP.

2. Experimental procedures

2.1. Materials

Chitosan ($M_r = 750\,000$, deacetylation degree: 83.5%) was obtained from Fluka Chemie AG

(Buchs, Switzerland). MT, DOS and pentasodium TPP were purchased from Sigma Chemical Co. (St. Louis, USA). All other materials used in the dissolution studies were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of chitosan microparticles

Chitosan microparticles containing MT were prepared by a capillary extrusion procedure (Shiraishi et al., 1993). Briefly, MT (1.5–4.5% w/v) was dispersed in a stirred solution of 2% w/v chitosan in 2% v/v acetic acid until a uniform dispersion was obtained. The microparticles were formed by dropping the bubble-free dispersion through a disposable syringe (with a nozzle of 1 mm inner diameter) into 20 ml of a gently agitated solution of the crosslinking agent (DOS or TPP). The dropping rate was 30 beads/min. The falling distance was 5 cm. The gelled microparticles were separated, unless otherwise noted, after a reaction time of 2 h, washed with deionized water and then air dried for 48 h. All batches were prepared in triplicate. A number of variables such as stabilization time, chitosan and crosslinking agent concentrations, as well as drug/polymer ratio were investigated for optimization of microparticle properties (Table 1).

2.2.2. Micromeritic properties of chitosan microparticles

The microparticles are characterized by their micromeritic properties, such as particle size, tapped bulk density, percentage compressibility index (% CI: a value useful in prediction of flowability) and true density (d). Diameter of dried microparticles was measured from the scanning electron micrographs. Density of microparticles was determined by immersing the microparticles in a 0.02% Tween 80 solution for 3 days in a metal mesh basket. The particles that are sunken after this process were used for density measurement as carried out by the displacement method using n-hexane as a non-solvent (Soppimath et al., 2001). The tapping method was used to calculate tapped densities and %CI according to the following equations:

$$\text{Tapped density} = \frac{\text{Mass of microparticles}}{\text{Volume of microparticles after tapping}}$$

$$\%\ \text{Compressibility index} = \left[1 - \frac{V}{V_o}\right] 100$$

where V and V_o are, respectively, the volumes of the sample after and before the standard tapping.

The tapping was carried out in a 10 ml measuring cylinder. After observing the initial volume of microparticles, the tapping was continued on a hard surface at a rate of 100 taps per min until no further change in volume was noted.

2.2.3. Preparation of DOS/chitosan solid complex

The chitosan solution (20 ml; 0.1%) was mixed with the DOS solution (20 ml; 0.1%). Dilute acetic acid (2% v/v) was used to prepare these samples. The sample solution was then incubated at 37 ± 0.5 °C for 24 h. After the removal of water from the sample solution, the remaining solid complex was dried under vacuum for 3 days at 37 ± 0.5 °C.

The solubility of the solid complex and intact chitosan were determined at room temperature using 0.02 g of polymer in 5 ml of various solvents, including: dimethylsulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), N-methylpyrolidone (NMP), tetrahydrofurane (THF), tetrahydrofurfuryl alcohol (THFFA), CH_2Cl_2, toluene and $CHCl_3$.

2.2.4. Instrumental analyses

Infrared spectra of the DOS/chitosan solid complex, intact chitosan and DOS were measured using an infrared spectrophotometer (IR-470, Shimadzu, Japan) with the KBr disk method. Differential scanning calorimetric (DSC) analyses were performed on the solid samples using a computer-interfaced Shimadzu calorimeter (Model DSC-50, Japan) with heating cycles of 0–400 °C. Samples (4–5 mg) were continuously heated at the rate of 10 °C/min under a constant flow of nitrogen gas. The maximum positions of melting endotherms were taken to be the melting points. The X-ray diffraction patterns of polymers were obtained using a Phillips X-ray diffratometer

Table 1
Influence of various formulation parameters on characteristics of chitosan microparticles

Variables	Values	Abbreviations [a]	Actual drug content (%w/w ± S.D.)	Incorporation efficiency[b] (%)	Duration of buoyancy (h)	$t_{50\%}$ (S.G.F.) (h ± S.D.)	K_o (%/h)	
							S.G.F.	S.I.F.
DOS concentration (%)[c]	1	A1	36.30 ± 1.14	54.46	> 18	1.75 ± 1.11	15.01 ± 2.85	11.13 ± 2.18
	2	A2	29.96 ± 0.49	44.95	> 12[g]	5.00 ± 0.088	8.26 ± 2.65	9.54 ± 1.01
	3	A3	27.57 ± 0.332	41.36	> 8[f]	3.80 ± 0.113	9.13 ± 0.764	10.74 ± 2.14
	4	A4	26.50 ± 0.933	39.75	No	3.15 ± 0.121	9.90 ± 0.850	11.53 ± 1.44
Drug/polymer ratio (w/w)[d]	1:1	B1	15.60 ± 1.76	31.20	> 8[f]	2.75 ± 1.21	10.92 ± 0.392	13.43 ± 0.035
	2:1	B2	29.96 ± 0.49	44.95	> 12[g]	5.00 ± 0.088	8.26 ± 2.65	9.54 ± 1.01
	3:1	B3	39.14 ± 1.48	52.19	> 18	4.75 ± 0.12	9.93 ± 0.184	12.11 ± 0.290
	4:1	B4	46.86 ± 1.53	58.58	> 18	4.35 ± 0.103	10.98 ± 0.202	11.81 ± 1.47
Chitosan concentration (%)[e]	1.5	C1	29.96 ± 0.49	44.95	> 12[g]	5.00 ± 0.088	8.26 ± 2.65	9.54 ± 1.01
	2	C2	33.60 ± 1.12	50.41	> 18	6.70 ± 0.072	7.12 ± 0.139	8.33 ± 0.679
	2.5	C3	39.82 ± 1.32	59.74	> 18	4.85 ± 0.081	8.80 ± 0.191	7.74 ± 0.525
TPP concentration (%)[c]	1	D1	45.20 ± 1.482	67.81	No	0.60 ± 1.06	53.57 ± 1.62	32.53 ± 0.064
	2	D2	40.70 ± 0.661	61.06	No	1.05 ± 1.23	39.86 ± 1.43	22.88 ± 1.15
	3	D3	41.22 ± 0.982	61.84	No	1.05 ± 0.98	40.24 ± 0.863	33.28 ± 0.679
	4	D4	41.26 ± 1.23	61.90	No	0.75 ± 0.872	42.24 ± 1.02	32.97 ± 0.898

No, microparticles did not float on S.G.F.
[a] A2, B2 and C1 are the same formulations.
[b] Incorporation efficiency (%) = actual drug content/theoretical drug content × 100.
[c] Chitosan concentration: 1.5%, drug/polymer ratio: 2:1.
[d] Chitosan concentration: 1.5%, 2% w/v DOS.
[e] Drug/polymer ratio: 2:1, 2% w/v DOS.
[f] ~30% of microcapsules floated on S.G.F. after a 8 h period.
[g] ~50% of microcapsules floated on S.G.F. after a 12 h period.

(Phillips generator PW-1710, Netherlands) with a Ni-filtered CuK_{α}-radiation at a scanning speed of 5 °C/min.

2.2.5. Assessment of drug incorporation into chitosan microparticles

About 50 mg of microparticles were digested in 100 ml of enzyme-free simulated intestinal fluid (S.I.F.: KH_2PO_4/NaOH buffer; pH 7.4) and extracted completely during a period of 24 h. The solution was filtered and the amount of MT was measured spectrophotometrically (Shimadzu, Double-Beam Spectrophotometer 150-02, Japan) at 278 nm. Each determination was made in triplicate.

2.2.6. Scanning electron microscopy (SEM)

The surface topography of the microparticles was examined using a scanning electron microscope (Jeol, JSM-5200, Japan, 15 KV). Samples were coated with gold film under vacuum using a sputter coater (SPI Sputter™ Coating Unit, SPI Supplies, Division of Structure Probe, Inc., PA, USA) and then investigated. Cross-sections were made in order to observe the core and internal structure of the microparticles.

2.2.7. Buoyancy test

The buoyancy of the microparticles was studied by using a water bath shaker with a shaking speed of 100 o.p.m. (oscillations per minute) at 37 ± 0.5 °C, soaking 50 microparticles in 100 ml of enzyme-free S.G.F. (HCl/NaCl solution containing 0.02% Tween 80; pH 1.2) or enzyme-free S.I.F. (KH_2PO_4/NaOH buffer containing 0.02% Tween 80; pH 7.4). Both the number of F microparticles (observed visually) and the F duration (the time during which the microparticles remain buoyant on the test solution) were then determined at fixed time intervals during a 18 h period. All the data were the average of at least three determinations.

2.2.8. Equilibrium swelling studies

A known weight (100 mg) of various chitosan microparticles without drug was placed in 500 ml of different solutions (distilled water, enzyme-free S.G.F. (HCl/NaCl solution; pH 1.2) and enzyme-free S.I.F. (KH_2PO_4/NaOH buffer; pH 7.4) and allowed to swell for the required period of time at 37 ± 0.5 °C using the USP dissolution apparatus with the dissolution basket assembly (Model DT-06, Erweka, Germany) at 50 rpm. The microparticles were periodically removed, blotted with filter paper and their changes in weight were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microparticles was recorded after a time period of 4 h and the swelling ratio (SR) was then calculated from the formula:

$$SR = \frac{(W_e - W_o)}{W_o}$$

where W_o is the initial weight of the dry microparticles and W_e is the weight of the swollen microparticles at equilibrium swelling in the media. Each experiment was repeated three times and the average value ± S.D. was taken as the SR value (Table 2).

2.2.9. In vitro release studies

The release of MT from chitosan microparticles (equivalent to 25 mg of drug) was investigated using the USP dissolution paddle assembly (Model DT-06, Erweka, Germany) with an agitation speed of 50 rpm in 250 ml of enzyme-free S.G.F. (HCl/NaCl solution containing 0.02% Tween 80, pH 1.2) and enzyme-free S.I.F. (KH_2PO_4/NaOH buffer containing 0.02% Tween 80, pH 7.4) at 37 ± 0.5 °C. At appropriate time intervals, 5 ml samples were withdrawn and assayed spectropho-

Table 2
Equilibrium SR of various drug-free chitosan microparticles (mean ± S.D.)

Formulation code[a]	Water	S.G.F. (pH 1.2)	S.I.F. (pH 7.4)
Ao1	2.83 ± 0.28	7.942 ± 0.18	0.89 ± 0.20
Co1	0.61 ± 0.10	0.800 ± 0.014	0.48 ± 0.11
Co2	0.74 ± 0.015	0.762 ± 0.016	0.30 ± 0.05
Co3	0.73 ± 0.11	1.23 ± 0.15	0.42 ± 0.18
Do1	0.402 ± 0.13	17.10[b] ± 0.21	0.71 ± 0.17
Do2	0.359 ± 0.054	27.86[b] ± 0.33	0.62 ± 0.28
Do3	0.325 ± 0.044	36.37[b] ± 0.42	0.54 ± 0.21

[a] Ao1, Co1–Co3 and Do1–Do3 are the same formulations as in Table 1 but without drug.

[b] The microspheres attained equilibrium swelling in 3 h.

tometrically at 278 nm. The UV-absorption with microparticles without drug in dissolution test conditions was also measured. All dissolution runs were performed in triplicate.

3. Results and discussion

3.1. Formation of chitosan microparticles loaded with MT

Chitosan, the cationic polyelectrolyte, forms gel with multivalent counterions (e.g. TPP) through the formation of intermolecular or intramolecular linkages by ionic interaction (Kawashima et al., 1985; Shiraishi et al., 1993). In this study, the droplets of chitosan solutions instantaneously formed gelled spheres by ionotropic gelation of the polysaccharide with the oppositely charged ions (DOS or TPP). DOS/chitosan water insoluble complexes are, thus, formed by the electrostatic combination of the amino group on the chitosan molecule and the carboxylate group on the anion (DOS) molecule (Fig. 1). The DOS/chitosan complex formation was confirmed employing IR spectroscopy. The IR spectrum of the resulting semisynthetic polymer was greatly different from that of the pure polymer (Fig. 2I (a and b)). The pattern of the new band, appearing in the range of 1670–1610 per cm, was assigned to a combination

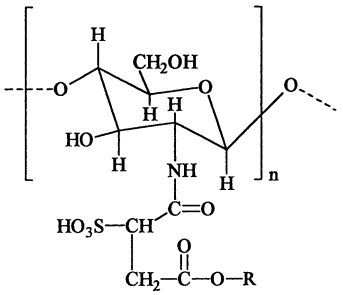
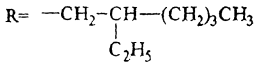


Fig. 1. Presumptive structure of the DOS/chitosan complex, covalent attachment of the DOS molecule was achieved by the constitution of an amide bond between a primary amino group of the polymer and the carboxylate group of DOS.



of the amidic C=O stretching and N–H bending (Fig. 2I (b)). The other non-reacted carboxylic ester group of DOS appeared as a sharper peak at 1736 per cm. The formation of an amide link between chitosan and DOS moiety was also supported by the absence of the broad, strong NH_3^+ stretching band of the salt in the 3100–2600 per cm region (Silverstein and Webster, 1998) (Fig. 2I (b)). It has been reported that the amino groups of chitosan are capable of interacting with an anionic polymers which has carboxyl groups such as sodium alginate and sodium polyacrylate by ionic bonding (Takahashi et al., 1990). Accordingly, it can be considered that the ionic bonding is a primary binding force for the complex formation between chitosan and DOS. The complex formation is also proven by using DSC. Under the experimental conditions, the DSC thermogram of pure chitosan has no characteristic endotherms; while that of the complex shows a sharp endothermic peak at about 250 °C (Fig. 2II (a and b)). This indicates the melting of a highly ordered polymeric structure that has an enthalpy of about 176.92 J/g. In addition, the pure polymer has a large exothermic decomposition peak at about 310 °C, whereas, that of the complex was a bit smaller and shifted to about 285 °C; further confirming that chitosan is not present in the sample in the free form (Fig. 2II (a and b)). The X-ray diffractograms of polymers indicated an overall amorphous pattern, but with different diffuse peaks in the region of $2\theta = 5–50°$ (Fig. 2III).

Surprisingly, the solubility studies in various organic solvents revealed that DOS/chistosan complex (C.F. pure chitosan) was soluble in polar aprotic solvents (e.g. DMSO, DMF, DMA or NMP), further supporting the absence of salt formation between chitosan and DOS. The resultant complex was also soluble in toluene and THFFA (polar protic solvent); partially soluble in THF, CH_2Cl_2 and $CHCl_3$. The good solubility of the complex in most of these tested solvents and its poor solubility in chitosan usual solvents (e.g. an acid aqueous medium) may be attributed to its high flexibility due to the acylation of the amino groups of chitosan by the inclusion of the DOS carboxylic acid residues into chitosan molecule.

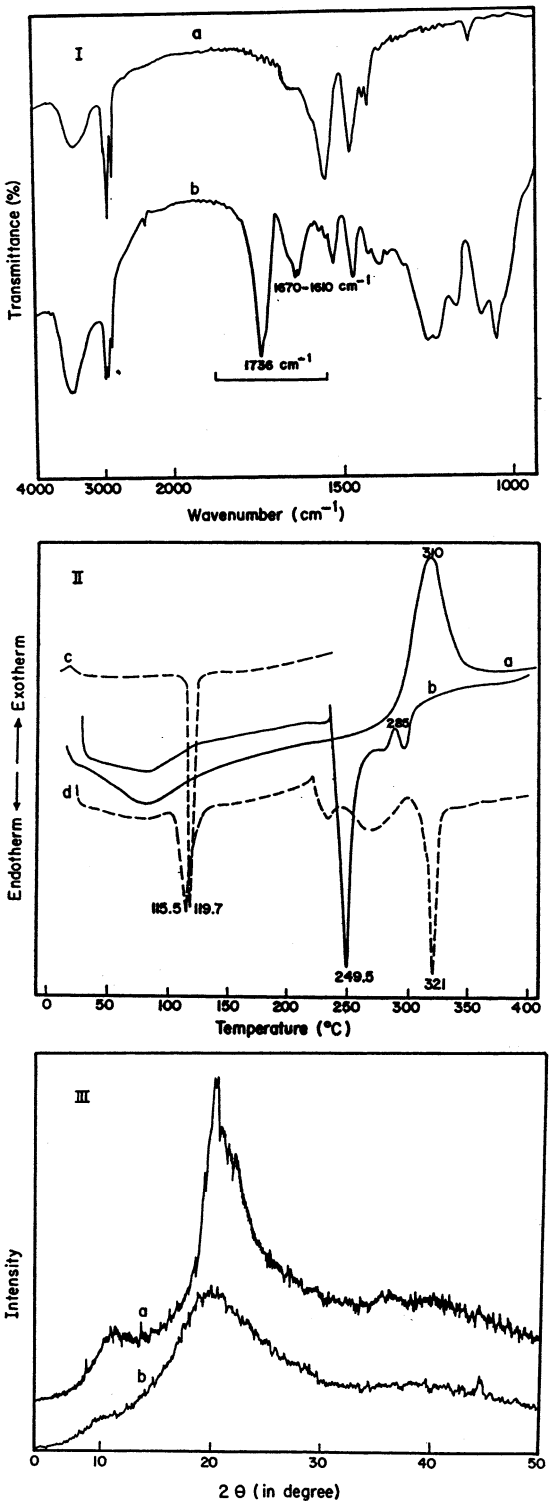


Fig. 2

To understand the physical state of the drug inside the microcapsules, DSC was also performed on pure drug and drug-loaded microcapsules (Fig. 2II (c and d)). The DSC thermogram of the loaded microcapsules revealed that an endothermic peak of melting of MT at about 120 °C was shifted to about 115 °C. This suggests that a crystalline form of MT existed in the microcapsules. The physical state of the drug in the microcapsules has an influence on the morphology and release kinetics of microcapsules (Soppimath et al., 2001).

3.2. Characterization of chitosan microparticles

3.2.1. Incorporation efficiency

Except for the low dose formulation B1, the drug loading efficiencies were found to be good and varied from 39.75 to 67.81% (Table 1). However, MT showed better entrapment efficiency (61.06–67.81%) in TPP/chitosan microspheres as compared with the drug entrapment efficiency in DOS/chitosan microcapsules. As seen in Table 1, the efficiency of drug incorporation was also influenced by the concentration of counter ions used; an increase in DOS or TPP concentration from 1 to 2% led to a marked decrease in drug loading efficiency. The entrapment of MT in DOS/chitosan microcapsules increased greatly from about 30 to about 47% as the drug/polymer ratio increased from 2:1 to 4:1 (formulations B2 and B4). Table 1 also shows a proportional increase in drug loading efficiency of DOS/chitosan microcapsules at enhanced concentrations of chitosan in the microcapsule preparative mixture (formulations C1–C3). The same result was also reported for indomethacin chitosan microspheres prepared by Orienti et al. (1996). They explained this effect by the increased viscosity of the microsphere preparative mixture which hinders drug migration towards the external preparative phase during microsphere preparation.

Fig. 2. Infrared spectra (I), DSC thermograms (II) and X-ray diffraction patterns (III) of: (a) chitosan alone and (b) DOS/chitosan complex. DSC thermograms of: (c) free MT and (d) MT-loaded DOS/chitosan microcapsules (drug/polymer ratio: 3:1) are also shown.

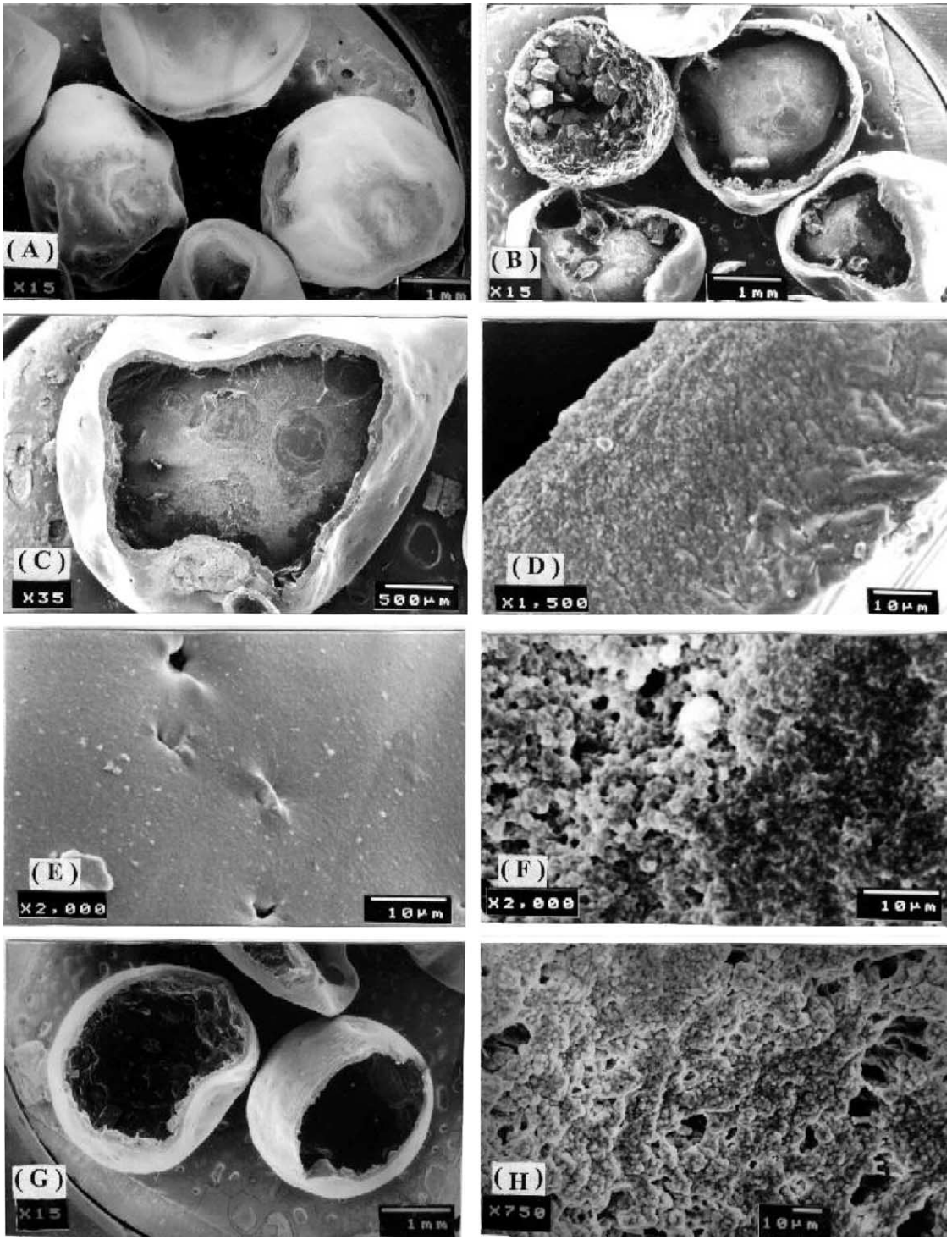

(A)
X15
1mm
(B)
X15
1mm
(C)
X35
500µm
(D)
X1,500
10µm
(E)
X2,000
10µm
(F)
X2,000
10µm
(G)
X15
1mm
(H)
X750
10µm

Fig. 3

3.2.2. Floating capacity

Buoyancy tests were performed in pH 1.2 and 7.4 buffers with 0.02% w/v of Tween 80 in order to simulate the surface tension of human gastric juice (35–50 mN/m^2) (Stithit et al., 1998; He et al., 1999). The results showed a tendency that the higher the DOS concentration, the poor the F properties of microcapsules (Table 1). In contrast, the F capacity was not influenced by the amounts of drug and chitosan added as nearly all of the hollow microcapsules remained buoyant after the buoyancy test period (18 h). The absence of the floatation lag time indicates that the original density of the microcapsules prior to matrix swelling in simulated biofluids was less than 1. In fact, the F process depends on the balance between the weight and the volume variations of the dosage forms. The volume increase causes the resultant-weight increase and then the dosage form floatation (Timmermans and Moës, 1990).

On the other hand, the F capacity of microcapsules, when formulated as single-unit dosage forms (e.g. capsules) depends on their micromeritic properties such as flowability and density. The data obtained revealed that the %CI values of the F microcapsules ranged between 11 and 22%, suggesting good flow characteristics of the prepared microcapsules (Soppimath et al., 2001). The tapped density values of the F microcapsules ranged from 0.188 to 0.241 g/cm^3, while their true densities ranged between 0.405 and 0.728 g/cm^3. Obviously the density values of the F microcapsules (< 1.000 g/cm^3) were less than that of the gastric fluid (~ 1.004 g/cm^3), thereby, implying that these microcapsules will have the propensity to exhibit an excellent buoyancy effect in vivo. However, TPP/chitosan microspheres exhibited compressibility values of 20–25%, higher tapped densities (0.527–0.708 g/cm^3) and true density values (1.01–1.33 g/cm^3); further supporting the NF nature of such microspheres.

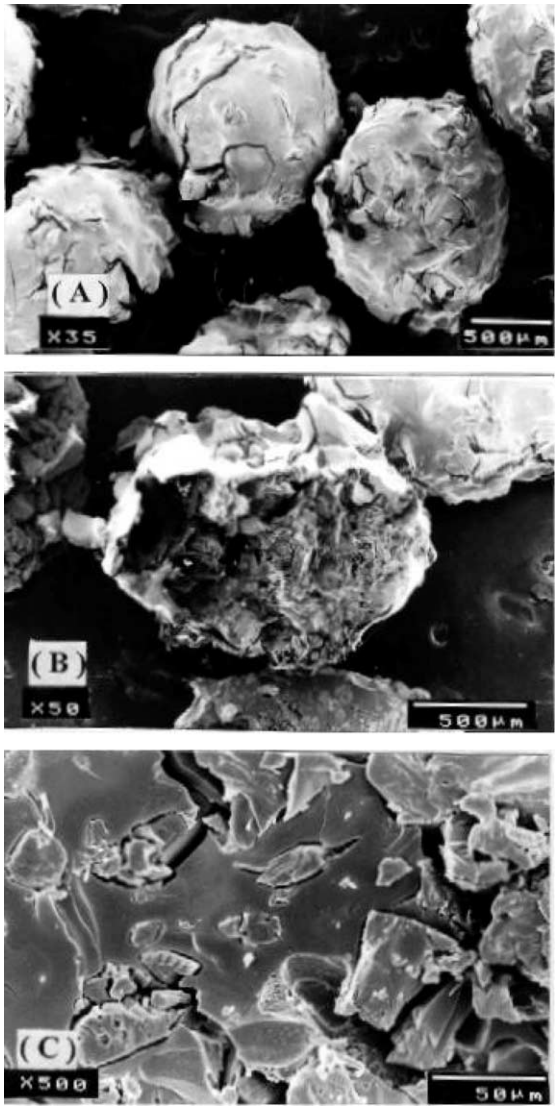


Fig. 4. SEM micrographs of NF drug-loaded TPP/chitosan microspheres (formulation D2) indicating (A) surface morphology; (B and C) cross-sections showing the internal structure of the microspheres.

3.2.3. Morphology

Fig. 3 represents the SEM micrographs of MT-loaded DOS/chitosan microparticles. The micro-

Fig. 3. SEM micrographs of F drug-loaded DOS/chitosan microcapsules prepared with varying chitosan concentrations. (A) microcapsules prepared with 2% chitosan (formulation C2); (B and C) cross-sections showing the hollow core and the outer shell of formulation C2; (D) a cross-section showing the internal structure of the shell of formulation C2; (E and F) morphologies of the outer surface and the internal surface of formulation C2, respectively; (G) a cross-section of the microcapsules prepared with 2.5% chitosan (formulation C3); (H) morphology of the internal surface of formulation C3.

graphs show regular shaped microparticles (2.4–2.95 mm in diameter) having an apparently homogenous and smooth surface with few wrinkles and inward dents due to the collapse of the microcapsule wall during the in situ drying process (Fig. 3A). The SEM micrographs of the microparticle cross-sections are shown in Fig. 3(B and C). It was observed that when chitosan is coagulated by DOS, microcapsule formation is verified, where a hollow core structure enclosed in a seamless outer shell is clearly defined. Drug crystals are clearly seen inside the hollow microcapsules. This indicates that there was not an intermixing of the wall material and the encapsulated drug (Fig. 3(A and B)). DSC studies confirmed this suggestion (Fig. 2II (c and d)). The region of the coating wall (magnified in Fig. 3D), shows that a compact wall with a thickness of about 65 μm and the least porosity appearance was obtained. The outer surface of the microcapsules was smooth and dense (Fig. 3E, × 2000). In addition, a few number of pinholes was seen on the surface of most microcapsules. There were also numerous micropores visible on the internal surface of the hollow spaces (Fig. 3F). The hollow microcapsules obtained with 2.5% concentration of chitosan exhibited fairly good spherical geometry (Fig. 3G), but the internal surface was porous and showed pinhole-like internal cavities throughout the solid matrices (Fig. 3H). Obviously, the findings indicate that 2% chitosan concentration was the most favorable to thicken the coating film of the microcapsules.

Fig. 4 illustrates that the use of TPP solution in coagulation of chitosan induces the formation of a microsphere structure in which a core is not defined. All batches of TPP/chitosan microspheres displayed essentially poor spherical particles (0.95–1.4 mm in diameter) with numerous surface cracks and pores compared with DOS/chitosan microcapsules (Fig. 3A and Fig. 4A). Ultrastructural characteristics of TPP microspheres were observed by cross-sections which showed that the interior of the microspheres had large open channels or interconnected pores and drug crystals embedded in the solid matrix (Fig. 4(B and C)).

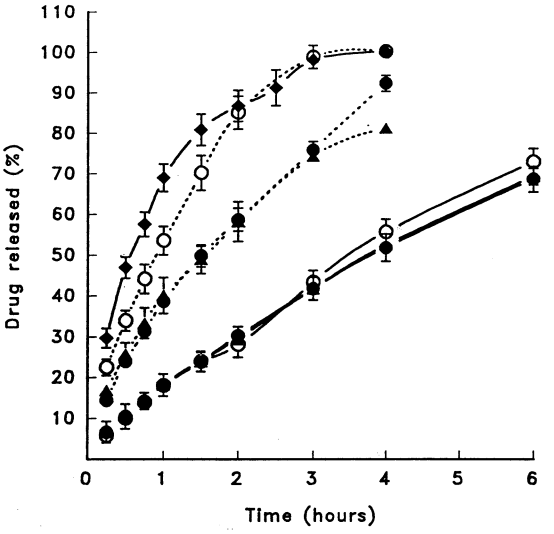


Fig. 5. Effect of crosslinking time on drug release from chitosan microparticles in S.I.F. (pH 7.4). DOS/chitosan microcapsules (solid line); TPP/chitosan microspheres (dotted line); crosslinking time: (○) 1 h; (●) 2 h; (▲) 24 h; (◆) free drug. Preparation conditions: crosslinking agent concentration: 2% w/v, 1.5% w/v chitosan; drug/polymer ratio: 2:1.

3.2.4. Swelling characteristics of chitosan microparticles

Microparticle swelling is influenced by the environmental pH, being generally greater at lower rather than higher pH value or water. The microspheres prepared with TPP reached the highest equilibrium SR (value = 17.10–36.37, depending on TPP concentration) at pH 1.2 in 3 h, whereas, their SR values were reduced to 0.61–0.78 in pH 7.4 medium (Table 2). On increasing TPP concentration, the microspheres became very soft and appeared as unformed and gelled material after immersion in pH 1.2 solution for 5 h, presumably due to an osmotic effect generated by the salt. A similar finding was reported on theophylline granules coated with a polyelectrolyte complex of TPP-chitosan (Kawashima et al., 1985). Table 2 revealed also that swelling in S.G.F. increased for DOS/chitosan microcapsules prepared from the lower concentration of DOS (1% w/v) (formulation Ao1, SR value = 7.942) or the higher percentage of chitosan (2.5% w/v) (formulation Co3, SR value = 1.23). Under these conditions, chitosan is present in excess (i.e. chitosan/DOS ratio > 1) and

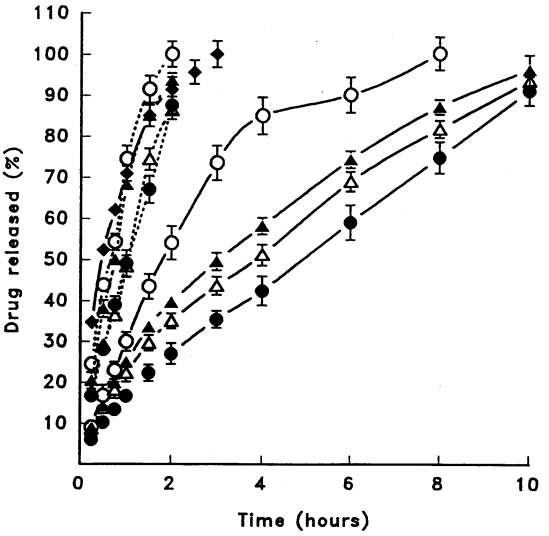


Fig. 6. Effect of crosslinking agent concentration on drug release from chitosan microparticles in S.G.F. (pH 1.2). DOS/chitosan microcapsules (solid line, notation A), TPP/chitosan microspheres (dotted line, notation D), crosslinking agent concentration: (○) 1% (A1, D1); (●) 2% (A2, D2); (△) 3% (A3, D3); (▲) 4% (A4, D4); (◆) free drug.

there will be an excess of NH_2 groups in the network. The protonation of any excess of the amino groups of the polysaccharide in stomach pH conditions accounts for this effect favoring the hydration and unfolding of the crosslinked polymeric structure and, therefore, its swelling (Orienti et al., 1996). In the case of the microcapsules prepared using 2% DOS and 1.5 or 2% chitosan (formulations Co1 and Co2; chitosan/DOS ratio ≤ 1), the lowest SR values(≈ 0.8) were obtained at pH 1.2 (Table 2) since any excess of the carboxylic acid residues within the polymer will exist predominantly as the poorly hydrophilic unionized species. These conditions resemble the optimum situation, where the ionic interaction between chitosan and DOS is maximal. Therefore, the hydrogels were able to retain gel integrity for more than 3 days of testing in S.G.F. due to the improved water resistance of DOS/chitosan films.

3.2.5. *Drug release from chitosan microparticles*

Fig. 5 depicts that the investigated crosslinking time factor (1–24 h) did not have significant effects on the release patterns of DOS/chitosan microcapsules in S.I.F. (pH 7.4). On the other hand, the release rate of MT decreased with increasing the crosslinking time of TPP/chitosan microspheres from 1 to 2 h. Therefore, the optimum crosslinking time chosen for chitosan microparticles was 2 h.

The drug release patterns of MT-loaded DOS/chitosan microcapsules and TPP/chitosan microspheres in S.G.F. (pH 1.2) were compared with the dissolution profile of the drug powder (Fig. 6). The release of MT from the DOS or TPP/chitosan microparticles was much slower than the dissolution of MT powder. Chitosan matrices were, thus, demonstrated to serve as barriers to the liberation of MT. Fig. 6 shows substantially great differences among the release profiles of DOS/chitosan microcapsules (formulations A1–A4) and TPP/chitosan microspheres (formulations D1–D4) in S.G.F. The DOS-crosslinked chitosan microcapsules remained largely intact (>3 days) and showed the most retarding effect on MT release with a $t_{50\%}$ (the time for 50% release of drug) of 1.75–5 h (depending on DOS concentration), compared with the conventional TPP/chitosan microspheres ($t_{50\%}$: 0.6–1.05 h, depending on TPP concentration) (Table 1 and Fig. 6). In S.G.F., the TPP-crosslinked microspheres exhibited approximately 95% MT release by the second hour and lost their integrity in this medium within 5 h, while values ranging from almost 27 to 40% release over an equivalent period were recorded for the microcapsules prepared with DOS (2–4% w/v). The slow release of drug from the F DOS/chitosan microcapsules was most likely due to the dense texture and low porosity of the coating film (Fig. 3), as well as poor wetting and hydration, so that the drug in the particles had poor contact with the dissolution medium. This result is in accordance with the report of He et al. (1999) on F chitosan microspheres crosslinked with glutaraldehyde. Therefore, it appears that differences in release rate could be explained by the nature of the crosslinked matrix formed, which could affect the hydration and swelling rate of the matrix during drug release and consequently the penetration of the solvent into the microparticles. The DOS-crosslinked chitosan matrix showed poor gel-forming ability and the smallest degree of swelling

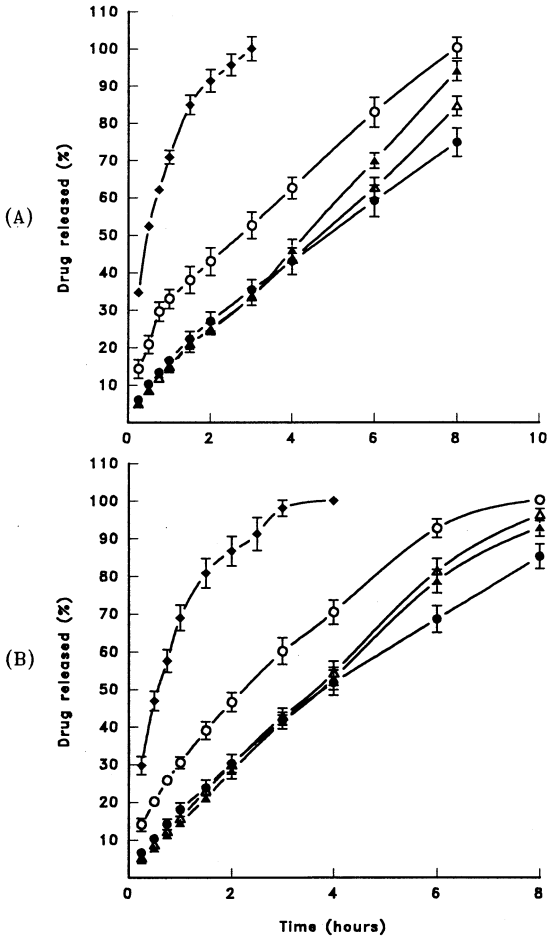


Fig. 7. Effect of drug/polymer ratio on drug release from F DOS/chitosan microcapsules in (A) S.G.F. (pH1.2) and (B) S.I.F. (pH 7.4). Drug/polymer ratio: (○) 1:1 (B1); (●) 2:1 (B2); (△) 3:1 (B3); (▲) 4:1 (B4); (◆) free drug.

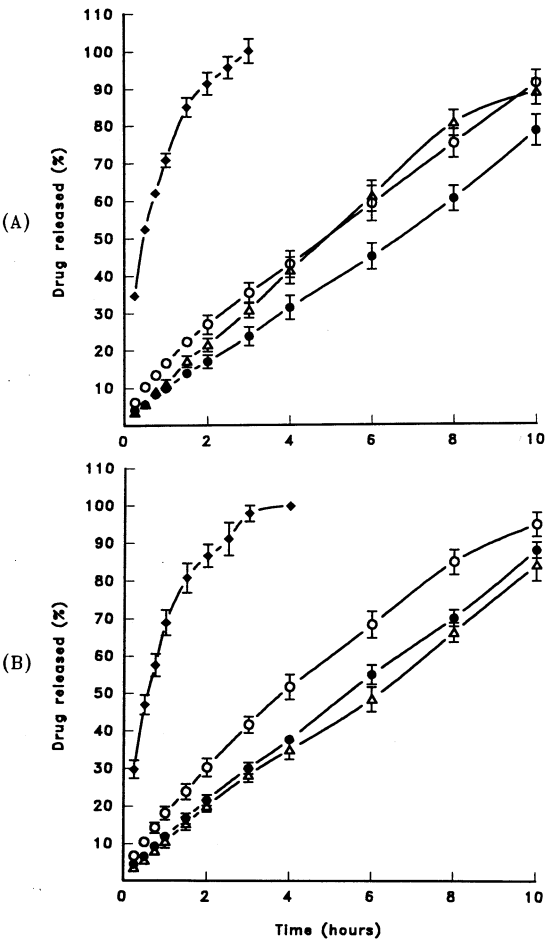


Fig. 8. Effect of chitosan concentration on drug release from F DOS/chitosan microcapsules in (A) S.G.F. (pH 1.2) and (B) S.I.F.(pH 7.4). Chitosan concentration: (○) 1.5% (C1); (●) 2% (C2); (△) 2.5% (C3); (◆) free drug.

in S.G.F. (SR values = 0.762–7.942), compared with TPP/chitosan matrix which showed rapid swelling in S.G.F. (SR values = 17.1–36.37) within 3 h (Table 2). Therefore, the use of a combination of DOS and TPP in microparticle preparation reduced significantly the drug release rates of the TPP/chitosan microspheres (data not shown).

The drug release profiles (in pH 1.2 buffers) from chitosan microcapsules prepared at various DOS concentrations are also shown in Fig. 6. Despite, the buoyancy of microcapsules prepared with 1% DOS (formulation A1, $d = 0.495$ g/cm^3), they exhibited the highest release rate in S.G.F. (K_o(%/h) = 15.01), with about 85% of the payload being released within 4 h. This may be attributed to their high drug content and enhanced swelling in S.G.F. (SR value = 7.942) (Tables 1 and 2). On the contrary, the release of drug was very much retarded with the F microcapsules prepared with 2% DOS (formulation A2, $d = 0.728$ g/cm^3; K_o(%/h) = 8.26). These microcapsules have released about 42% of the encapsulated drug within 4 h, while about 51 and 58% of total drug content have been released, respectively, from the poorly F microcapsules prepared with 3 and 4% DOS (formulations A3 ($d = 0.910$ g/cm^3) and A4 ($d =$

1.12 g/cm^3), respectively) (Table 1). It can be suggested, as an explanation, that 2% DOS generates more dense polymer crosslinking (Fig. 3) effective in impeding the swelling of the microcapsules (SR value ≈ 0.80, Table 2) and consequently slowing down drug release rate in the first 4 h. Surprisingly, the results obtained with TPP/chitosan formulations (D1–D4) prepared at various TPP concentrations revealed also that the lowest drug release rate was obtained with 2% concentration of TPP (Table 1 and Fig. 6).

Table 1 and Fig. 7 show that the rate of release was influenced by the initial drug loading of the DOS/chitosan microcapsules. Thus, the total amount of drug released in S.G.F. within 8 h was about 100, 75, 85 and 95% of the entrapped drug for the microcapsules prepared with 1:1, 2:1, 3:1 and 4:1 drug/polymer mixtures, respectively (Fig. 7). Obviously, a 1.3 to 1.4-fold (approximately) increase in the release rate (depending on the pH of the release medium) was observed when the drug loading was reduced from 30 (drug/polymer ratio: 2:1) to 15.6% (drug/polymer ratio: 1:1) (Table 1 and Fig. 7). Similar data were also reported for crosslinked chitosan microspheres containing griseofulvin (Thanoo et al., 1992).

The effects of chitosan concentration on drug release characteristics are given in Table 1 and Fig. 8. Evidently, The $t_{50\%}$ value (S.G.F.) was markedly prolonged from about 5.0 to about 6.7 h when chitosan concentration was increased from 1.5 (formulation C1) to 2% (formulation C2). In addition, only 60–70% of drug loaded (depending on the pH of the release medium) was released from formulation C2 in 8 h and the drug was not completely released from these microcapsules during the test period (10 h) (Fig. 8). However, faster release rate ($t_{50\%} \approx 4.85$h) was observed with microcapsules prepared using 2.5% chitosan concentration. This may be ascribed to a probably lower crosslinking degree due to the higher viscosity of the preparative phase of the microcapsules hindering the crosslinking process, as explained by the increased swelling of the polymeric network at this chitosan concentration (Table 2). These data showed similarity with the report of Orienti et al. (1996) concerning indomethacin-loaded chitosan microspheres.

The drug release rate also depended on the pH of the dissolution medium employed, as shown in Table 1 and Figs. 7 and 8. The drug release from most of the DOS/chitosan microcapsules was found to be slightly higher in S.I.F. than S.G.F. inspite of their low swelling in both the fluids (Table 2, formulations Co1–Co3). This finding might be interpreted in the light of the fact that chitosan, which is soluble in media of low pH, will experience electrostatic repulsion in media pH of 6.0 or higher leading to changes in polymer chain conformation. The resulting polymer chain shrinkage manifests as increased membrane pore size, which accounts for the relatively higher drug release in S.I.F. A similar tendency has been reported for drug release from chitosan/alginate microcapsules (Okhamafe et al., 1996). On the contrary, the drug release rate of TPP/chitosan microspheres in pH 1.2 medium was greatly enhanced as compared with that at pH 7.4. The results could be interpreted in terms of the degree of swelling of microspheres which depended on the pH of the medium (Table 2). At low pH, the degree of swelling increased, which loosened the surface crosslinked layer and increased the void volume in the texture. In addition, as evidenced by

Table 3
Values of r^2 from release data of some formulations for different models of mechanisms of drug release (pH 1.2)

Model	B2	C1	C2	C3	D2
Zero-order	0.9950	0.9972	0.9992	0.9993	0.9986
First-order	0.9230	0.9846	0.9813	0.9616	0.9373
Higuchi	0.9518	0.9854	0.9686	0.9630	0.9864

Data for the time interval from the first to the 8 h.

the SEM micrographs in Fig. 4, the more porous internal structure and loose texture of the TPP/chitosan coating films might enhance the swelling and drug release also. These results indicate the predominance of the swelling effect over the effect of electrostatic repulsion hypothesis on drug release from TPP/chistosan microspheres.

The release data of some representative formulations were fitted to models representing zero-order, first-order and Higuchi's square-root of time (Higuchi, 1963) processes. As seen in Table 3, the highest determination coefficients (r^2) were obtained with zero-order kinetics, thereby, indicating a time-independent release process. The observed constant release suggests that after swelling, the coating film thickness of the microcapsule was almost constant and the saturated drug concentration inside the microcapsule was also held constant during the drug release test.

4. Conclusions

Novel hollow microcapsules containing MT were prepared under mild conditions using a simple and inexpensive gel matrix. In fact, the use of DOS to form F chitosan gel sacs insoluble under stomach pH conditions was not reported in the literature before for the preparation of controlled-release drug carriers. Overall, the buoyant DOS/chitosan microcapsules produced provide a promising gastroretentive drug delivery system to deliver MT with a sustained and near zero order release rate. The F microcapsules prepared with 2% chitosan, drug/polymer ratio of 2:1 (or 3:1) and 2% DOS might be the best for the sustained-release preparation of MT. Drug release properties of these microcapsules can be easily altered by changing the process factors. In contrast, conventional NF microparticles processed with TPP had poor morphological features and exhibited significantly faster drug release rates than those prepared with DOS. Therefore, the F microcapsules obtained may be an interesting candidate for maximizing the therapeutic effectiveness of drugs included in the controlled release dosage forms.

Acknowledgements

The author would like to thank Dr Abdel-Nasser El-Shorbagi (Professor of Pharmaceutical Organic Chemistry, Assiut University) for the help in the interpretation of IR results.

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