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RESEARCH PAPER

Different Molecular Weight Chitosan Microspheres: Influence on Drug Loading and Drug Release

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ABSTRACT

Influence of chitosan molecular weight on drug loading and drug release of drug-loaded chitosan microspheres was studied. Chitosans of 70,000 (LC), 750,000 (MC), and 2,000,000 (HC) molecular weight were employed alone or as mixtures (HC/LC 1:1–1:2 w/w). Ketoprofen (ket) was chosen as the model drug to be encapsulated. Microspheres characterized by different theoretical polymer/drug ratios were prepared (2:1, 1:1, 1:2 w/w). Satisfactory ket contents were obtained for all batches of chitosan microspheres with the theoretical polymer/drug ratio 1:2 w/w; microspheres made of HC/LC (1:2 w/w) were characterized by good drug content and encapsulation efficiency independent by polymer/drug ratio. Prepared chitosan microparticulate delivery systems can modulate ket release within 48 hr. Microspheres consisting of HC/LC (1:2 w/w) were the most suitable formulation in controlling drug release.

INTRODUCTION

Chitosans are natural, biocompatible, and biodegradable polymers that are very useful for their potential pharmaceutical and medical applications (1).

Among the pharmaceutical applications, the formulation of chitosan microparticulate delivery systems seems to be particularly advantageous for oral, mucosal, and parenteral administration (2–4). Microparticulate systems based on chitosan can be manufactured by various techniques: ionotropic gelation, emulsion-phase separation, dry-in-oil method, multiple-emulsion method, and spray-drying (5–8). Drug diffusion from chitosan matrices could be effectively controlled by chemical crosslinking, using a dialdehyde such as glutaraldehyde (9), or heat treatment (10).

Members of the chitosan family differ in terms of molecular weight and degree of deacetylation. In this

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work we attempted to develop drug-loaded microspheres based on chitosans and mixtures of chitosans of different molecular weights in order to investigate the influence of the polymeric composition of the microspheres on drug content and on drug release. An anti-inflammatory drug, ketoprofen (ket), was chosen as model drug to be encapsulated.

MATERIALS

Different molecular weight chitosans (HC, MC, and LC) were purchased from Fluka (Buchs, Switzerland): HC (MW 2,000,000, deacetylation degree 83%), MC (MW 750,000, deacetylation degree 83.5%), and LC (MW 70,000, deacetylation degree 87.4%).

Ket was supplied by Carlo Erba (Milan, Italy). Span 20 was supplied by Atlas (Essen, Germany). Glacial acetic acid, methanol, ethanol, isopropanol, mineral oil, petroleum ether (Carlo Erba), sodium cacodylate, and osmium tetroxide (Fluka) were analytical grade. The distilled water used was prepared in our laboratory.

METHODS

Rheological Measurements

Viscosity measurements were performed with a Rotovisco RV12 rotational cup and bob viscosimeter (Haake, Karlsruhe, Germany) equipped with an NV sensor system. Analyses were carried out on 0.5% chitosan solutions in 2% acetic acid that had settled for 2 hr (Table 1).

Preparation of Microspheres

Microspheres were prepared according to a modified dry-in-oil emulsion method (11).

Different types of chitosan or mixtures of chitosans (Table 2) at fixed concentration (0.5 % w/v) were solubilized in 2% acetic acid solution. Preliminary experiments indicated the most suitable polymer solution concentration to obtain microparticles with good morphological characteristics for all types and mixtures of chitosans employed. In order to obtain microspheres with different theoretical polymer/drug ratio, ket was dissolved at different concentrations (1, 2, and 4% w/v) in ethanol and added to chitosan solution in a 1:4 v/v ratio (Table 2). The dispersion obtained was dropped in paraffin oil containing 2% w/v Span 20. The ratio between the two phases of the emulsion was 1:13 v/v. The emulsion was performed at room temperature (RT) and under continuous stirring (Ultraturrax T25 S25N10G, Ika Labortechnik, Staufen, Germany) at 9500 rpm for 15 min; then it was heated at 60°C (heating rate 5°C/10 min) and stirred with a Vibromixer E1 (Chemap, Volketswil, Switzerland) at 50 vibrations/sec under reduced pressure by a vacuum pump for 19 hr until the solvent was evaporated completely.

The microsphere suspension was rinsed with petroleum ether; the microspheres were collected by centrifugation and dried under vacuum for 48 hr.

Table 2 lists all the batches of ket-loaded chitosan microspheres produced. Each batch was produced in triplicate.

Characterization of Microspheres

Optical Microscopy

The microspheres were characterized by optical microscopy, using a standard microscope (Carl Zeiss, Oberkochen, Germany) equipped with camera (Reichert, Wien, Austria). The dried microspheres were suspended in mineral oil and then photographed at 256× magnification.

In order to evaluate the rate of swelling of microspheres with different polymeric composition, samples of each batch were suspended in phosphate buffer pH 7.4 (USP 23) and immediately photographed; the same samples were allowed to settle for 15 min, and were then microscopically observed and photographed.

Table 1

High Shear Viscosity of Chitosan Solutions (0.5% w/v) in 2% Acetic Acid

Polymer type	LC	MC	HC	HC/LC (1:2, w/w)	HC/LC (1:1, w/w)
Molecular weight ($\times 10^3$)	70	750	2000	–	–
High shear viscosity (mPa·s)	39.0	19.7	40.7	37.5	39.4

Table 2

Theoretical Composition of Chitosan Microspheres

Batch	Type of Chitosan	Ket Solution (%)	Theoretical Polymer/Drug (w/w)
1	HC	1.0	2:1
2	HC	2.0	1:1
3	HC	4.0	1:2
4	MC	1.0	2:1
5	MC	2.0	1:1
6	MC	4.0	1:2
7	LC	1.0	2:1
8	LC	2.0	1:1
9	LC	4.0	1:2
10	HC/LC (1:2 w/w)	1.0	2:1
11	HC/LC (1:2 w/w)	2.0	1:1
12	HC/LC (1:2 w/w)	4.0	1:2
13	HC/LC (1:1 w/w)	1.0	2:1
14	HC/LC (1:1 w/w)	2.0	1:1
15	HC/LC (1:1 w/w)	4.0	1:2

Thin-Section Electron Microscopy

Microspheres were visualized by thin-section electron microscopy using a JEM 1200 EXII transmission electron microscope (Jeol, Tokyo, Japan). Microspheres were fixed with 4% glutaraldehyde (in 0.1 M sodium cacodylate, pH 7.4) for 2.5 hr at room temperature, then post-fixed with 1% osmium tetroxide (pH 7.4) for 1.5 hr at 4°C. The samples were then rinsed with phosphate buffer saline (USP 23) and allowed to set in the buffer overnight. The samples were dehydrated with 50, 95, and 99.8% ethanol at 30-min sequences. They were incubated in epoxypropane for 30 min and then the specimens were embedded in a mixture of epoxypropane: Epon 812 1:3 w/w for 2–5 hr. Sections were cut from embedded specimens with a Ultracut Ultramicrotome (Reichert), at a thickness of 2.5 μm.

Particle Size Analysis

Particle size analyses were performed on samples of microspheres suspended in isopropanol alcohol using a HIAC/ROYCO light blockage apparatus (AM Instruments, Desio, Italy).

The analyses were carried out at six channels, between 2 and 50 μm. The results are the average of three analyses.

Microsphere Drug Content

The chitosan microspheres loaded with ket were dissolved in an HCl 0.1 N/ethanol (1:1 v/v) mixture. The amount of drug loaded was determined by analysis with a model 9010 HPLC (Varian, Milano, Italy). A Lichrosorb RP18 column, 25 × 0.4 cm (Merck Bracco, Milano, Italy) was employed in conjunction with a UV detector at 254 nm. The mobile phase was a methanol/0.04% CH_3COOH mixture (65:35 v/v). An ethanolic solution of ket (10 μg/ml) was employed as the external standard.

In Vitro Drug Release Studies

In vitro release tests were carried out on all batches of ket-loaded chitosan microspheres. Amounts of microspheres containing about 1 mg of ket, as well as a reference sample made of the same amount of pure drug, were suspended in 100 ml phosphate buffer pH 7.4 (USP 23) containing ethanol (10% v/v).

The microsphere suspensions contained in capped Erlenmeyer flasks were agitated in a shaker incubator (Isco Italia, Milan, Italy) at 30 strokes/min for 72 hr at 37°C.

At scheduled time intervals, the agitation was stopped, the microsphere suspensions were allowed to settle for 5 min, and 400 μl of the dissolution medium was collected and analyzed for drug content. Dissolution medium was replaced with fresh medium.

All dissolution tests were run in triplicate and mean values are reported.

RESULTS AND DISCUSSION

The preparation method produced well-formed microspheres with good morphological characteristics for all batches prepared as shown (see Fig. 1). Ultrastructural characteristics of all batches of microparticulate systems were observed by thin-section electron microscopy: ket is embedded into the chitosan matrix (Fig. 2).

Particle size analyses revealed that the batches of microspheres were characterized by size distributions with $D_{50\%}$ (median diameter) ranging between 2.8 and 4.5 μm and $D_{90\%}$ (diameters of 90% of the particles are smaller than the reported value) between 8 and 15 μm. Chitosan molecular weight, polymeric composition and polymer/drug ratio in the microspheres did not influence particle size characteristics.

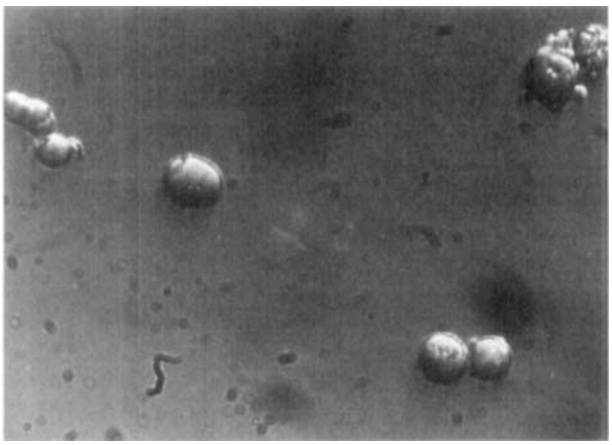

Figure 1. Optical microscopy photograph of ket-loaded chitosan microspheres (batch 13).

Drug content and encapsulation efficiency of ket-loaded chitosan microspheres are reported in Table 3. For each chitosan employed, the efficiency of drug encapsulation into microspheres improved, thus decreasing theoretical polymer/drug ratio. The highest encapsulation efficiency (17.4%) was obtained for LC microspheres (batch 9). The HC/LC (1:2 w/w) mixture exhibited high ket encapsulation efficiency independent of the polymer/drug ratio (11.8–15.9%).

Table 3

Actual Drug Contents and Encapsulation Efficiencies of Chitosan Microspheres

Batch	Actual Ket Content (% w/w)	Encapsulation Efficiency[a] (% w/w)
1	1.88	5.63
2	4.30	8.61
3	8.20	12.30
4	1.45	4.36
5	6.14	12.28
6	9.11	13.67
7	1.32	3.95
8	4.72	9.49
9	11.54	17.44
10	4.84	14.51
11	5.89	11.79
12	10.59	15.89
13	1.37	4.12
14	3.48	6.97
15	5.53	12.05

[a]Encapsulation efficiency = theoretical drug content/actual drug content × 100.

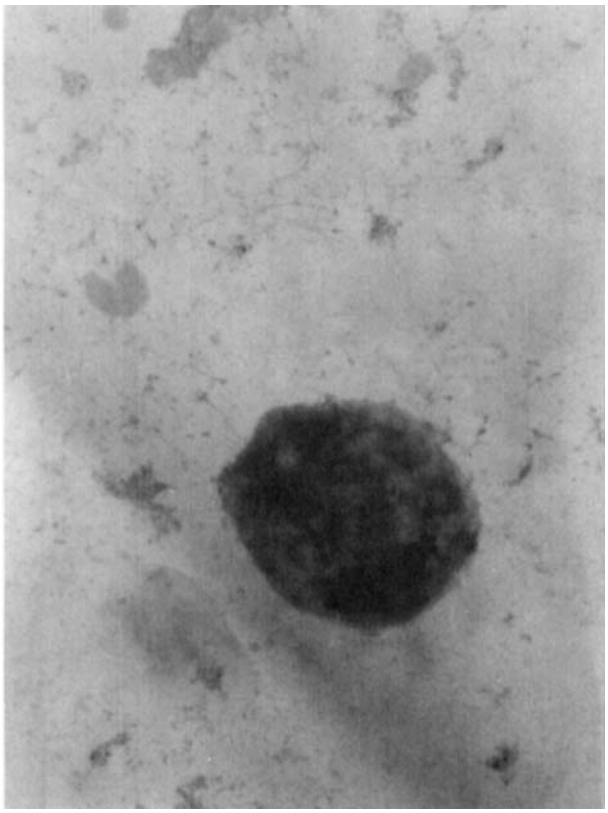

Figure 2. Thin-section electron micrograph of ket-loaded chitosan microspheres (batch 3).

In vitro ket release from chitosan microspheres with different polymeric compositions was investigated. A reference sample of pure ket dissolved completely in 15 min. All batches of ket-loaded microspheres showed the most significant differences in drug release in the first 4 hr and completely released the drug in 48 hr.

HC microsphere dissolution profiles are shown in Fig.3: a slower drug release is achieved for batch 1

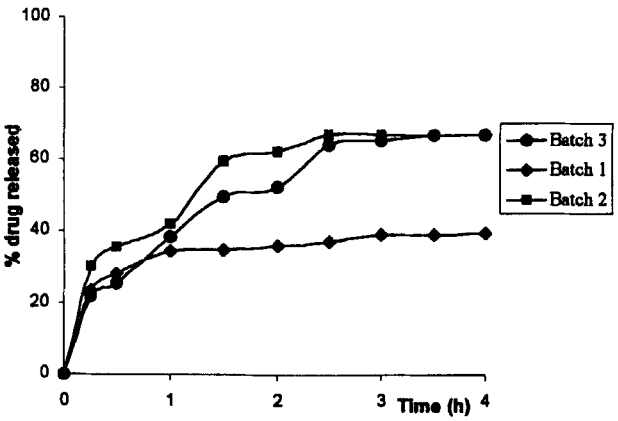

Figure 3. Ket release profiles from HC microspheres.

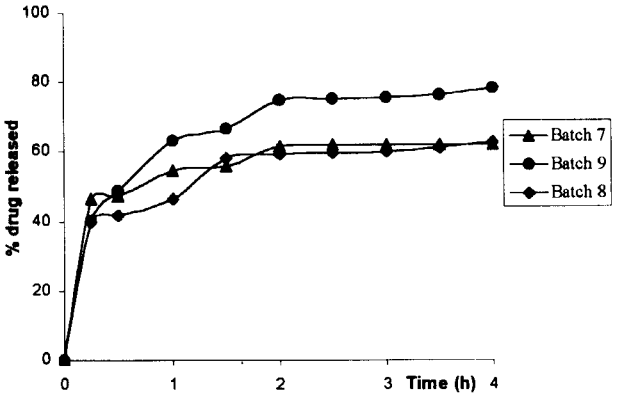

Figure 4. Ket release profiles from LC microspheres.

(1.88% drug content), and batches 2 and 3 (drug contents of 4.3 and 8.2%, respectively) show faster and superimposible dissolution profiles. A better control on drug release was obtained for microspheres made of the theoretical polymer/drug ratio 2:1 w/w and the lowest drug content. For MC and LC microspheres, ket release profiles did not seem critically dependent on the polymer/drug ratio employed; Fig. 4 shows, as an example, ket dissolution profiles from LC microspheres.

Drug release profiles from microspheres constituted by different molecular weight chitosans and with the same theoretical polymer/drug ratio were compared. HC and LC microspheres with the theoretical polymer/drug ratio 1:1 and 1:2 w/w are characterized by insignificantly different ket dissolution profiles (unreported data). Drug release profiles from HC, MC, and LC microspheres with 2:1 w/w theoretical polymer/drug ratio are shown in Fig. 5: after 15 min HC, MC, and LC microspheres have, respectively, released about 23, 64, and 46% of drug encapsulated. The fastest ket dissolution profile from MC microspheres, obtained for all polymer/drug ratios employed, can be explained by the swelling rate of this polymer in the dissolution medium. Optical microscope photomicrographs of dried microspheres and microspheres suspended in buffer for 15 min confirmed that MC microspheres swell and dissolve very quickly with respect to HC and LC microspheres [Figs. 6(a,b)]. This anomalous behavior is confirmed also by the viscosity measurements made for chitosan solutions at the same concentration (Table 1): independent of molecular weight, the high shear viscosity of MC acetic solution is

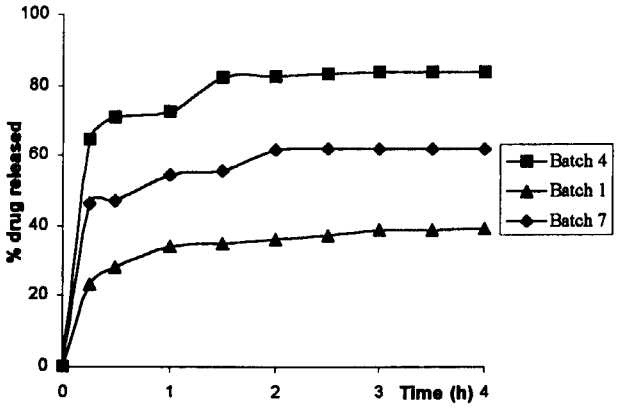

Figure 5. Ket release profiles from HC, MC, and LC microspheres made of theoretical polymer/drug ratio 2:1 (w/w).

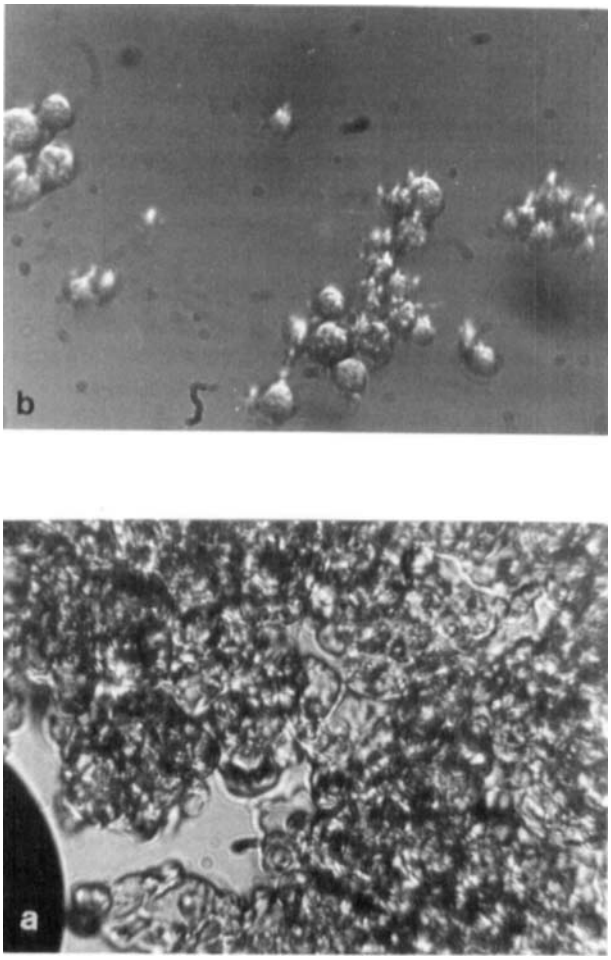

Figure 6. Optical microscopy photographs of ket-loaded MC microspheres (batch 4) suspended in phosphate buffer and immediately photographed (a), or after 15 min (b). (a) Microspheres maintain their spherical form; (b) microspheres appear as unformed, gelled material.

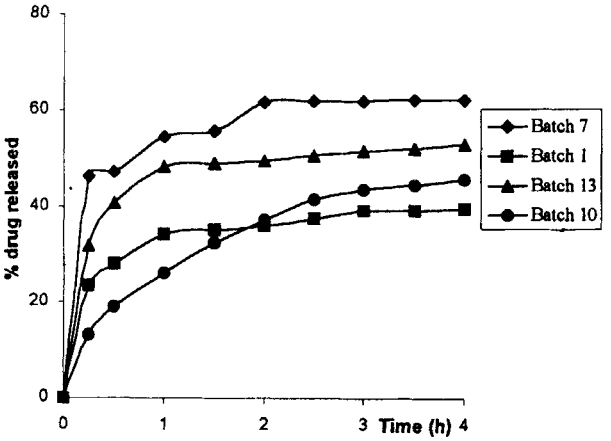

Figure 7. Ket release profiles from microspheres made of HC, LC, or mixtures and produced by a theoretical polymer/drug ratio 2:1 (w/w).

very low with respect to the solutions of HC, LC, and mixtures.

For this reason, only HC and LC polymeric mixtures (1:1–1:2 w/w) were employed in order to modulate ket release from chitosan microspheres.

Figure 7 compares ket dissolution profiles from chitosan microspheres made of HC, LC, and mixtures (theoretical polymer/drug 2:1 w/w). Microspheres constituted by HC/LC (1:1 w/w) show drug dissolution profile ranging between the release profile of HC and LC microspheres. HC/LC (1:2 w/w) microspheres present the lowest burst effect and the most regular ket dissolution profile.

CONCLUSIONS

Chitosans with MW ranging between 70,000 and 2,000,000 are suitable polymeric carriers for ket-loaded microparticles which can modulate drug release within 48 hr. The use of appropriate mixtures of chitosans with different molecular weight (i.e., HC/LC 1:2 w/w) produces ket-loaded microspheres characterized by a good drug content and a drug encapsulation efficiency independent of the polymer/drug ratio; furthermore, this polymeric composition induces the most regular drug release profile, thereby decreasing significantly the burst effect.

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