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Research paper

Chitosan and chondroitin microspheres for oral-administration controlled release of metoclopramide

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Abstract

This study investigated the usefulness of chitosan and chondroitin sulphate microspheres for controlled release of metoclopramide hydrochloride in oral administration. Microspheres were prepared by spray drying of aqueous polymer dispersions containing the drug and different amounts of formaldehyde as cross-linker. Drug release kinetics were investigated in vitro in media of different pH. Chondroitin sulphate microspheres scarcely retarded drug release, regardless of cross-linker concentration and medium pH, and were thus not further characterized. Chitosan microspheres prepared with more than 15% formaldehyde (w/w with respect to polymer) showed good control release (more than 8 h), and release rates were little affected by medium pH. Release from chitosan microspheres prepared with 20% formaldehyde was independent of pH, suggesting that this may be the most appropriate formulation. The size distribution of the chitosan microparticles was clearly bimodal, with the smaller-diameter subpopulation corresponding to microsphere fragments and other particles. Electron microscopy showed the chitosan microspheres to be almost-spherical, though with shallow invaginations. The kinetics of drug release from chitosan microspheres were best fitted by models originally developed for systems in which release rate is largely governed by rate of diffusion through the matrix.

Keywords: Chitosan; Chondroitin sulfate; Microspheres; Metoclopramide; Spray drying

1. Introduction

Metoclopramide hydrochloride is a potent antiemetic and prokinetic, effective even for preventing emesis induced by cancer chemotherapy [1]. It is also used in the treatment of certain disorders of the digestive tract, including gastric stasis and gastroesophageal reflux [2]. It is highly water-soluble and is rapidly absorbed after oral administration [3]. Its short half-life means that it must be administered in three or four doses of 10–15 mg per day, so that the development of controlled-release forms would clearly be advantageous. Furthermore, its most important drawback is that it may have secondary effects in the central nervous system if plasma levels markedly exceed therapeutic levels. Controlled release might be expected to ameliorate such problems, by reducing the height of the post-administration plasma peak.

The use of microsphere systems favours homogeneous and reproducible drug absorption, since the microspheres distribute throughout the digestive tract [4]. In the present study, we evaluated the use of such systems for administration of metoclopramide hydrochloride.

The polymers evaluated for the microencapsulation were a chitosan hydrochloride and a chondroitin sulphate (Fig. 1). Both are of natural origin, and are biodegradable, biocompatible and generally recognised to be bioadhesive [6–9]. Chitosan (obtained by deacetylation of chitin, the polymer forming the exoskeleton of crustaceans) is a cationic polymer that has been proposed for use in microsphere systems by various authors [10–13]. Chondroitin sulphate (obtained from mammalian connective tissue) is an anionic polymer whose possible use in microsphere systems is still controversial [6,14].

Microspheres were produced by spray drying, which is a rapid high-yield technique that is applicable at industrial scale and that enables small microspheres to be obtained from hydrogel-forming polymers [5].

The microspheres obtained were characterised by a series of pharmaceutical properties, including microencapsulation yield, microsphere morphology and size distribution, and drug release kinetics in media of different pH.

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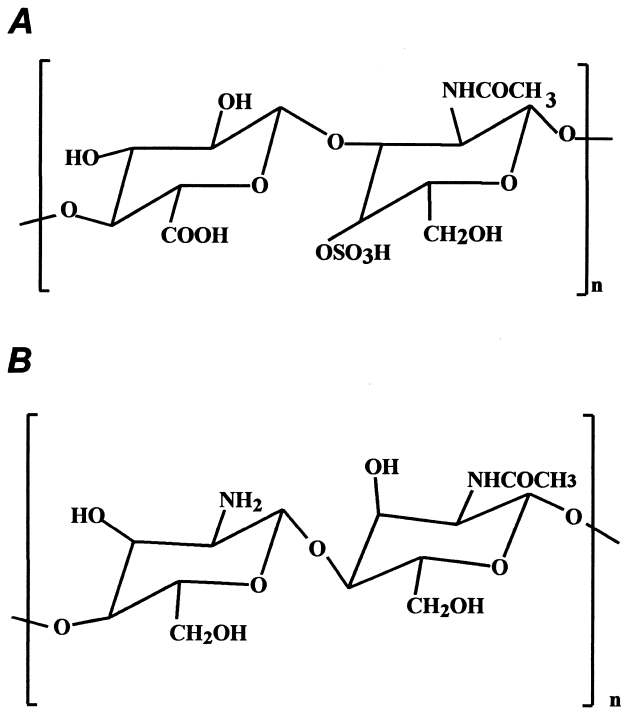

Fig. 1. Chemical structures of (a) chondroitin sulphate and (b) chitosan.

2. Materials and methods

2.1. Reagents and chemicals

Low- to medium-molecular-weight chitosan hydrochloride showing 20–30% deacetylation (Seacure® C1 210) was purchased from Pronova Biomedical (Norway). Chondroitin sulphate A (approximate molecular weight 45 000 Da.) and formaldehyde were from Sigma Chemical Co. (USA). Metoclopramide hydrochloride was from Roig Farma (Spain). All other reagents (hydrochloric acid, PBS, sodium chloride and sodium hydroxide) were from Probus (Spain).

2.2. Preparation of microspheres

A pale, yellow, almost transparent aqueous dispersion of polymer (1% w/w) containing 0.2% (w/w) metoclopramide hydrochloride was spray-dried (Buchi 190 mini spray drier, Switzerland) with a flow rate of 5 ml/min, inlet air temperature of 130°C and outlet air temperature of 70°C. In order to obtain insoluble matrices capable of slowing drug release, formaldehyde was incorporated into the dispersions at various concentrations (0.15, 0.20, 0.25 or 0.50% w/w) as cross-linking agent; glutaraldehyde could not be used, since it interfered with metoclopramide hydrochloride determination. The dispersions used for microsphere preparation thus contained 5, 20, 25 or 50% of cross-linking agent (expressed with respect to dry weight of polymer). No changes were observed in the polymer dispersion after the addition of metoclopramide hydrochloride and formaldehyde.

Table 1
Drug loading onto chitosan microspheres, and encapsulation yield estimates (mean ± SD, $n = 12$)

Formulation (% of cross-linking agent)	Drug content (%)	Encapsulation efficacy (%)
0	12.40 ± 0.09	70.75 ± 0.71
15	11.60 ± 0.12	65.6 ± 1.03
20	11.32 ± 0.08	63.8 ± 0.71
25	11.23 ± 0.04	63.3 ± 0.36
50	12.10 ± 0.15	68.8 ± 1.24

2.3. Drug loading

Metoclopramide hydrochloride levels were determined on the basis of absorption at 272 nm using a diode-array spectrophotometer (Hewlett Packard 8452A, Germany). The amount of drug present in the microspheres was determined after vigorous shaking in water for 24 h, which is sufficient to ensure complete release.

2.4. In vitro drug release

Drug dissolution assays were performed with a slight modification of apparatus no. I from USP 23 (Prolabo appa-

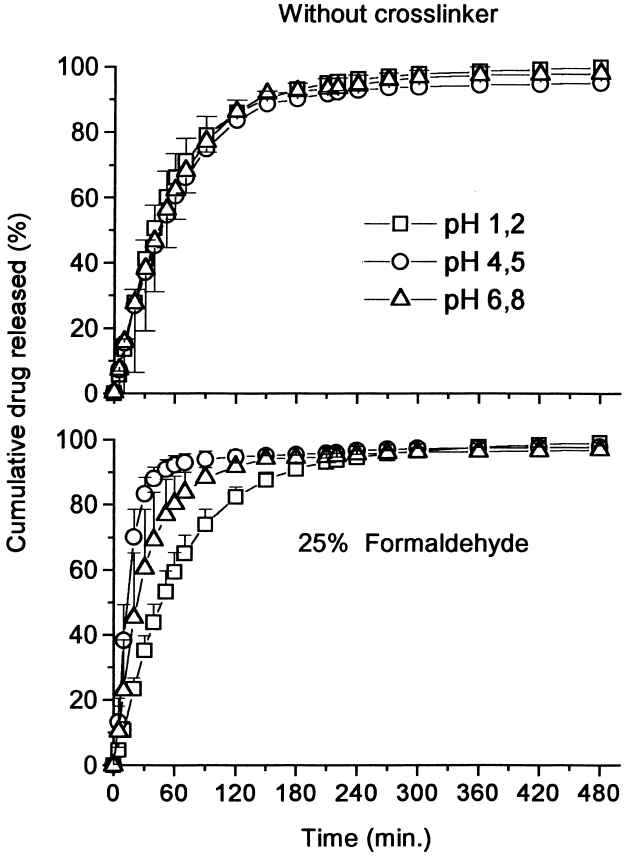

Fig. 2. Metoclopramide hydrochloride release profiles from chondroitin sulphate microspheres prepared without cross-linker and with 25% cross-linker (formaldehyde).

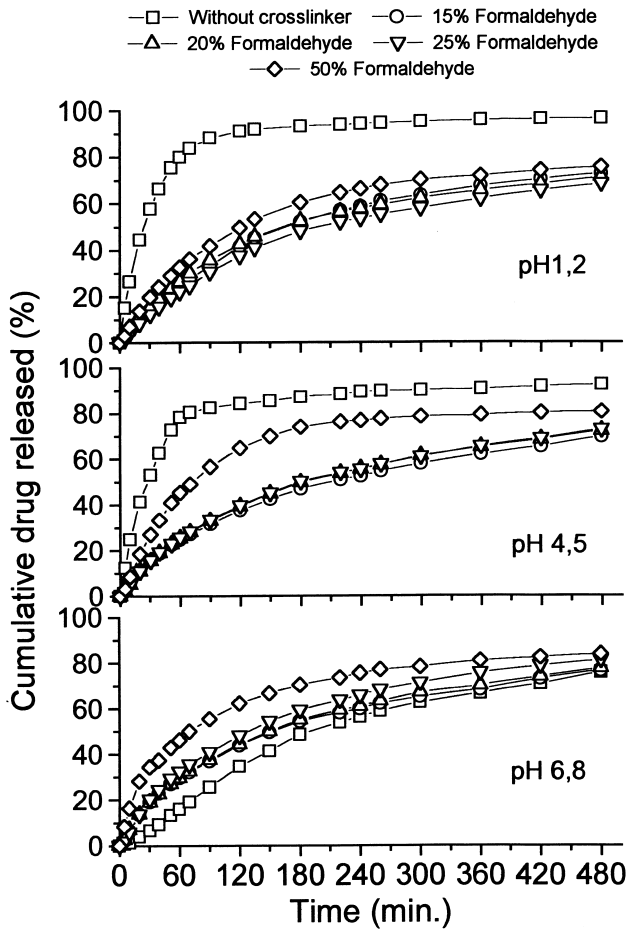

Fig. 3. Metoclopramide hydrochloride release profiles from chitosan microspheres prepared with different amounts of cross-linker (formaldehyde, % w/w with respect to polymer) in media of different pH.

ratus connected to a Hewlett Packard 8452A spectrophotometer). Specifically, the baskets used were slightly larger (34 mm in diameter, 44 mm high), and were covered with 5-μm-mesh nylon gauze. This design allows the microspheres to swell, and at the same time prevents them escaping to the medium. The dissolution media used were enzyme-free artificial gastric juice, PBS pH 4.5, and pH 6.8, in accordance with USP 23 guidelines. Medium temperature was 37°C, with stirring at 90 rev./min. Six replicates of each assay were performed. Standard drug-release models were fitted to the experimental data by least-squares procedures, as performed by the program Origin v. 5.0 (Microcal Software Inc., USA).

2.5. Size and morphology studies

Microspheres were examined with a scanning electron microscope (JSM R840 JEOL, Japan), after sputter coating with gold. Size (Feret diameter) and circularity [4πarea/(perimeter^2)] were estimated by light microscopy (Olympus BX60, Japan), using the image-analysis program PC-Image v. 2.1 (Foster Findlay Associated Ltd., UK). Theoretical frequency distributions were fitted to the observed normal particle-size distributions by least-squares procedures as performed with Origin v. 5.0.

Table 2
Circularity ($C = 4\pi$ area/(perimeter^2)) of cross-linked chitosan microspheres in the dry state and in aqueous suspension (means ± SD for 1200 light-microscopy measurements)

Formulation (% of cross-linking agent)	Circularity	
	Powder	Aqueous suspension
15	0.963 ± 0.0789	0.975 ± 0.0233
20	0.974 ± 0.0445	0.976 ± 0.0243
25	0.974 ± 0.0567	0.978 ± 0.0196
50	0.973 ± 0.0393	0.981 ± 0.0194

2.6. Statistical analysis

Analyses of variance, and subsequent Newman–Keuls multiple comparisons tests, were performed with the program Sigmastat (Jandel Corporation, USA), with $\alpha = 0.05$.

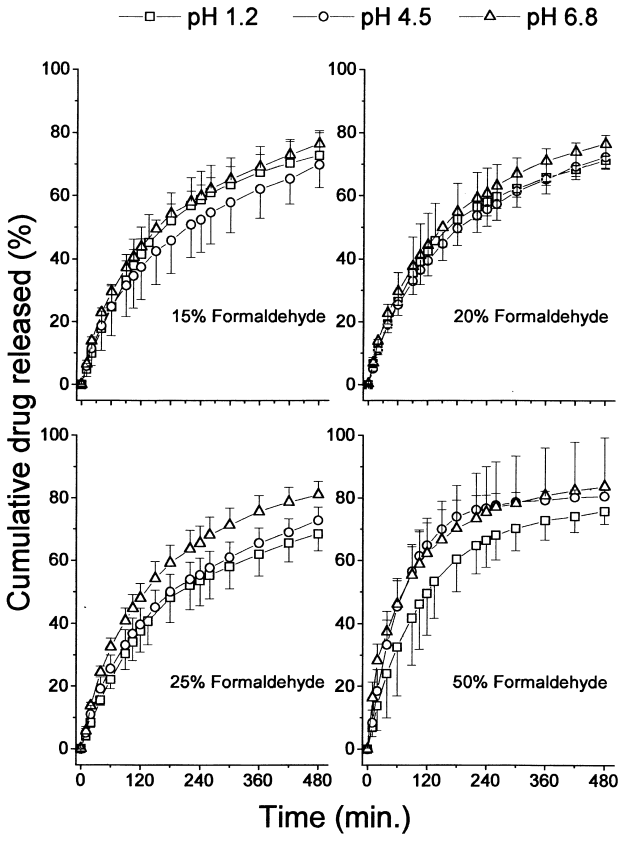

Fig. 4. Metoclopramide hydrochloride release profiles from chitosan microspheres prepared with different amounts of cross-linker (formaldehyde, % w/w with respect to polymer) in media of different pH.

Table 3
Size (Feret diameter) distribution statistics for dry chitosan microspheres ($n = 1200$ particles). See also Fig. 7

Formulation (% formaldehyde)	Subpopulation	Centre (μm)	Area under the curve (μm*%)	Mean (μm)
15	1	1.156	5.547	2.707 ± 0.900
	2	3.168	19.768	
20	1	1.018	2.311	2.612 ± 0.731
	2	2.758	23.077	
25	1	1.450	1.314	2.922 ± 0.629
	2	2.998	23.674	
50	1	1.230	2.543	2.912 ± 0.681
	2	3.066	27.954	

3. Results and discussion

3.1. Chondroitin sulphate microspheres

Spray drying of dispersions of chondroitin sulphate A gave microspheres with a diameter ranging from 1–6 μm, and a mean Feret diameter of 2.4 μm (SD = 1.1 μm) The mean drug content of these microspheres was 13.04 ± 0.3% w/w, giving a drug encapsulation yield of 67% ($n = 12$). Yield was not significantly affected ($\alpha = 0.169$) by formaldehyde content in the dispersion.

Chondroitin sulphate microspheres prepared without formaldehyde as cross-linking agent showed very rapid drug release in dissolution assays in all three media, due to solubilisation of the polymer and consequent disaggregation of the microspheres. Similarly, the drug was rapidly released from chondroitin sulphate microspheres prepared with formaldehyde: neither percentage released after 2 h nor percentage released after 8 h was significantly affected by formaldehyde content (see also Fig. 2). Also, differences were neither observed with higher concentrations of formaldehyde nor when using glutaraldehyde as cross-linking agent (data not shown). This suggests that inclusion of an aldehyde did not cause sufficient cross-linkage, probably because the chondroitin sulphate does not have enough free amino groups react with these cross-linking agents. In view of this finding we did not further characterise microspheres made with this polymer. Future studies should be aimed at identifying appropriate agents (e.g. carbodiimides) or procedures for obtaining adequately cross-linked chondroitin sulphate microspheres based on the reaction with the carboxylic groups.

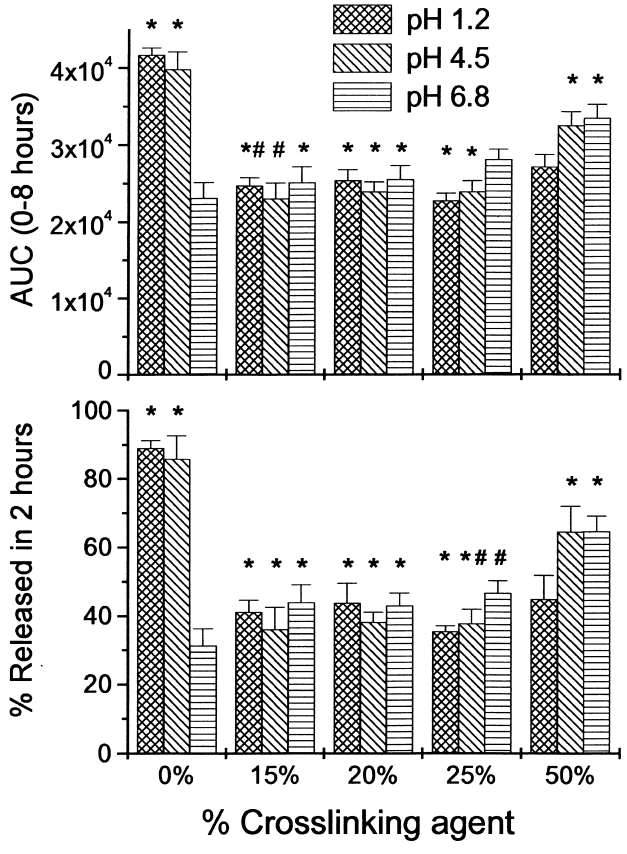

Fig. 5. Drug-release statistics (area under the curve, AUC_{0-8h}, and % released after 2 h) for each formulation ($n = 6$; scale bars show standard deviations). Within each formulation, means with the same letter do not differ significantly at the 5% level.

3.2. Chitosan microspheres

Chitosan microspheres obtained by the same method have a mean Feret diameter of 2.8 μm, with encapsulation yields ranging from 63 and 72% (Table 1). Analysis of variance indicated that formaldehyde content had a significant effect on encapsulation yield, and subsequent multiple comparisons tests indicated that all pairwise differences were significant at the 5% level. The origin of the differences is difficult to explain. No conclusions can be derived from this data because encapsulation yields do not follow any homogeneous tendency and the real content of formaldehyde or its structure in the microspheres (as cross-linking or polymerised as polyformaldehyde) is not known. On the other hand, the differences in encapsulation yield, although statistically significant, are too small (7.5–0.5%) to be considered from a practical point of view.

Chitosan microspheres prepared without formaldehyde as cross-linking agent showed scant capacity to retard drug release, except at pH 6.8, in which release is delayed for more than 8 h (Fig. 3). This latter result is attributable to the low solubility of this polymer at pH 6 or higher, so that little gelation occurs.

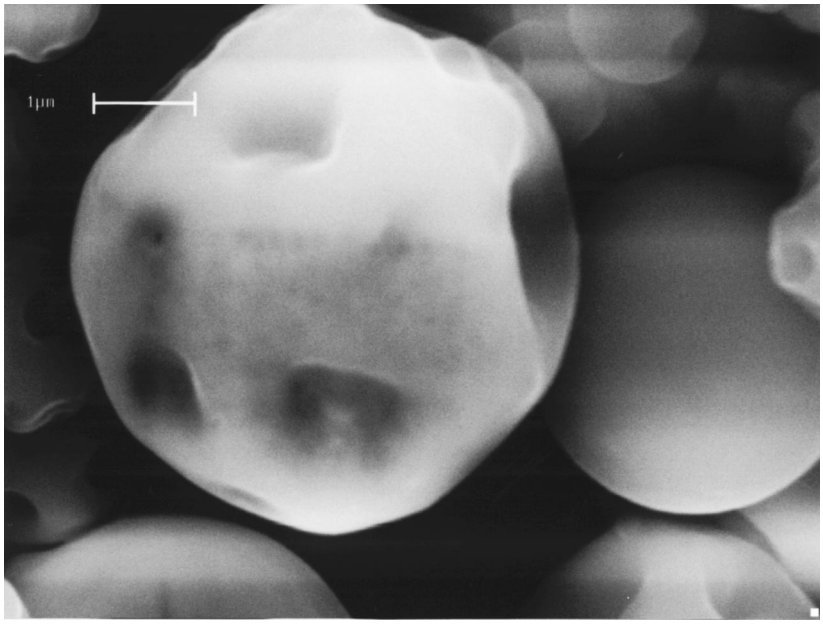

Fig. 6. Scanning electron micrograph (× 11 500) of a chitosan microsphere obtained with 20% formaldehyde as cross-linking agent.

In contrast, chitosan microspheres prepared with formaldehyde delayed release for more than 8 h in all three media tested, indicating efficient cross-linkage. Formaldehyde concentrations of less than 15% (with respect to polymer dry weight) did not effectively delay release (results not shown). Analysis of variance (factors formaldehyde content and medium pH) indicated that area under the cumulative drug release/time curve (AUC_{0-8h}) was significantly affected by formaldehyde content, while subsequent multiple comparisons tests indicated that AUC_{0-8h} differed significantly between the extremes, but not among intermediate formaldehyde contents (0, 15, 20, 25, 50%). The results obtained with microspheres prepared with 50% formaldehyde (expressed with respect to polymer weight; equivalent to more than 31% of microsphere weight) indicate excessive cross-linkage, giving rise to excessively rigid structures that swell less slowly, so that drug release is less effectively retarded. In fact, the formaldehyde polymerises at high concentration, so the microspheres would be formed by a poly-formaldehyde/chitosan copolymer. The poly-formaldehyde is more hydrophilic, rigid and unable to swell, modifying the drug release properties and probably the yield of drug encapsulation.

In addition, and as can be seen from Fig. 4, the release profiles obtained hardly varied with medium pH. This means that release will be little affected by pH variations in the gastrointestinal tract, favouring slow and homogeneous release. However, statistical analyses (see Fig. 5) indicate that release was entirely independent of medium pH only for microspheres prepared with 20% formaldehyde, suggesting that this is the optimal formulation in this regard.

The scanning electron microscope images of chitosan microspheres (Fig. 6) in all cases show a smooth surface. The fact that the particles are not perfectly spherical is probably attributable to the rapid drying process used in their preparation, giving rise to numerous invaginations. However, circularity estimates based on light microscopy measurements were close to one (Table 2), probably because the invaginations are less apparent in light-microscope images. In aqueous suspension, rehydration of microspheres occurs, causing the polymer to gelate and the microsphere to swell, thus 'correcting' the invaginations.

Table 4
Size (Feret diameter) distribution statistics for chitosan microspheres in aqueous suspension ($n = 1200$ particles). See also Fig. 7

Formulation (% formaldehyde)	Subpopulation	Centre (µm)	Area under the curve (µm*%)	Mean (µm)
15	1	1.741	10.551	5.134 ± 2.068
	2	5.843	38.241	
20	1	1.724	11.828	5.158 ± 2.244
	2	6.0865	36.156	
25	1	2.568	7.299	5.992 ± 1.668
	2	6.353	40.321	
50	1	2.526	10.343	5.217 ± 1.475
	2	5.683	36.574	

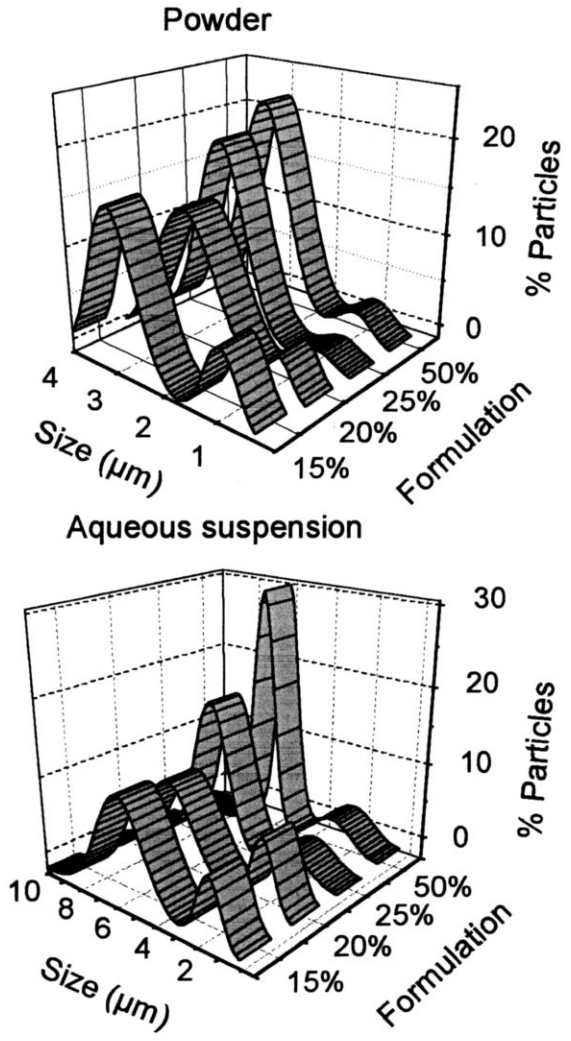

Fig. 7. Size (Feret diameter, μm) distribution of the particles obtained by the chitosan microsphere preparation process. Results are shown for particles in aqueous suspension and in the dry state.

Size distributions were also estimated on the basis of light microscopy. As can be seen from Fig. 7, the distribution size of the dry microspheres was in all cases bimodal. The largest subpopulation comprising particles with a mean Feret diameter of about 2–4 μm and the smaller subpopulation less than 2 μm. Bimodal size distributions of this type are common in microsphere studies: the large particles are

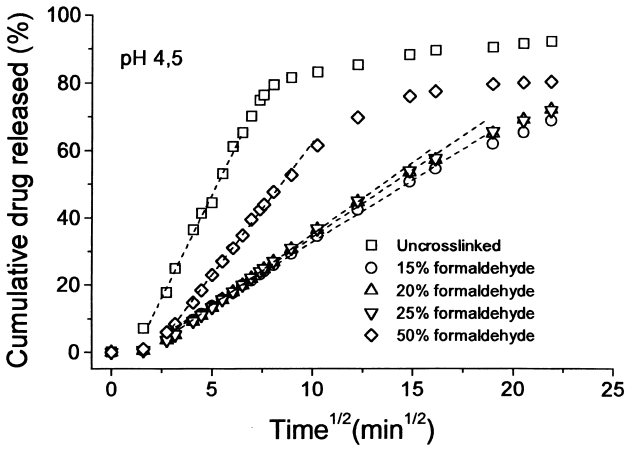

Fig. 8. Drug release profiles from chitosan microspheres prepared with different amounts of cross-linker (formaldehyde, % w/w with respect to polymer) in medium at pH 4.5, showing release predicted by fitting the Higuchi equation (dashed lines). See also Table 5.

microspheres, and the smaller particles fragments of non-encapsulated material and fragments of the microspheres themselves [15]. Fig. 7 also indicates that the proportion of smaller particles was lower when higher concentrations of cross-linking agent were used (see Table 3), indicating more homogeneous and mechanically stronger microspheres.

Since swelling of microspheres due to polymer gelation can be expected to have marked effects on drug release, we also characterised the size distribution of microspheres in aqueous suspension. Bimodal distributions were maintained in all cases, with particles of both subpopulations being almost twice as large as in the dry state (Fig. 7, Table 4). The mean swelling factor ($d_{swelled}/d_{dry}$) was between 1.8 and 2.0 for all four formulations studied, suggesting that microsphere structure varies little between formulations.

Finally, we characterised drug release kinetics by fitting standard release equations (zero-, first- and second-order; Higuchi equation, etc.) to the experimental data[16]. Following exclusion from the analysis of the first phase (<10% of drug released) and the last phase (>60% of drug released), the best fit was obtained with the Higuchi equation

$$M_t/M_0 = K\sqrt{t}$$

Table 5
Apparent diffusion constants for metoclopramide hydrochloride in Chitosan microspheres, as estimated by fitting the Higuchi model to the experimental data

% of cross-linker	pH 1.2		pH 4.5		pH 6.8	
	K ± SD (%/min)$^{0.5}$	R	K ± SD (%/min)$^{0.5}$	R	K ± SD (%/min)$^{0.5}$	R
0	13.812 ± 0.099	0.999	12.253 ± 0.340	0.996	5.272 ± 0.072	0.998
15	4.922 ± 0.051	0.999	3.7045 ± 0.038	0.999	4.544 ± 0.033	0.999
20	4.833 ± 0.046	0.999	4.031 ± 0.049	0.998	4.639 ± 0.025	0.999
25	4.594 ± 0.038	0.999	4.285 ± 0.0349	0.999	5.151 ± 0.060	0.998
50	5.534 ± 0.022	0.999	7.877 ± 0.102	0.998	5.954 ± 0.138	0.994

The results obtained with this equation are shown in Fig. 8 and Table 5. The equation was first proposed by Higuchi to describe the release of a solute from a flat surface, not from a sphere; nevertheless, the good fit obtained suggests that release rate is strongly dependent on the rate of diffusion of the drug through the cross-linked chitosan gel. The fits obtained using the equations of Guy et al. [17] (developed to describe diffusion from a sphere) were marginally worse (coefficients of determination 0.935–0.991) but still very good, confirming the importance of this mechanism.

4. Conclusions

Spray drying has been shown to be a fast, simple and reliable method for obtaining small microspheres from hydrogel-forming polymers. Formaldehyde-cross-linked chitosan microspheres are potentially useful for the administration of metoclopramide hydrochloride, since they offer drug release that is sustained for more than 8 h and that is practically independent of pH. The principal mechanism of release appears to be diffusion of the drug through the chitosan gel.

Acknowledgements

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