Preparation, characterization and *in vitro* drug release of poly-ε-caprolactone and hydroxypropyl methylcellulose phthalate ketoprofen loaded microspheres

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Ketoprofen was encapsulated within poly-ε-caprolactone (PCL) and hydroxypropyl methylcellulose phthalate 50 (HPMCP50) microspheres (MS). Scanning electron microscopy (SEM) studies showed spherical particles without surface crystal formation and differential scanning calorimetry (DSC) supported these results. MS of PCL or HPMCP50 had a mean particle size of 10.7 ± 2.2 and 10.9 ± 2.0 µm respectively, whereas a mixture of these polymers increased the MS particle size to 30 µm. Greater incorporation efficiencies were found for HPMCP50 MS ($98.1 \pm 0.7\%$). MS of PCL and HPMCP50 mixtures showed a decreased drug entrapment as the amount of PCL was increased (96.0 ± 0.2 for 25% PCL, 95.6 ± 1.8 for 50% PCL, 80.2 ± 0.7 for 75% PCL and 78.9 ± 9.0 for 100% PCL). Size exclusion chromatography (SEC) studies revealed a weak interaction between ketoprofen and PCL and some polymer degradation was found during HPMCP50 MS storage, probably by breaking of the phthalic anhydride bond to be anyhydroglucose backbone. Four types of cryoprotectors (glucose, trehalose, mannitol and sorbitol, at 5 and 10% W/V) and two freezing conditions (-196 and $-20°C$) were evaluated in freeze-drying studies. For HPMCP50, the sizes of MS after reconstitution of liophylizates were nearly the same as the initial ones. For PCL MS only, those formulations with sorbitol or glucose at 10% and frozen at $-196°C$ showed acceptable results. In contrast to the rapid release rate of ketoprofen from PCL MS as a result of carrier porosity (80% released within 15 min), the release from HPMCP50 MS could be controlled by means of pH (40% released in the first 15 min in simulated gastric fluid and nearly 100% ketoprofen delivered in the same time in simulated intestinal fluid).

Keywords: Ketoprofen, polycaprolactone, hydroxypropyl methylcellulose phthalate, biodegradable microspheres, *in vitro* release.

Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID), derived from propionic acid, that inhibits cycloxygenase and is denoted as a type I prostaglandin synthetase inhibitor. As other drugs which display the same therapeutic effect, it shows adverse reactions, mainly in the gastrointestinal (GI) mucosa, when administered by the oral route (Martindale, 1989). Furthermore, the conventional

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dosage forms of ketroprofen exhibit large variability in gastric-emptying times and short biological half-lives which results in a multiple-dose administration regimen. Based on these facts, some work has been focused to obtain oral sustained-action dosage forms (Marcolongo *et al.* 1981, Hagen 1982, Houghton *et al.* 1984a) in order to achieve a decrease of gastrointestinal side-effects as well as a once-daily administration (Houghton *et al.* 1984b) which would lead to a better patient compliance. Also transdermal-delivery systems has been investigated as a non-conventional route of drug administration, however, the barrier proper-ties of intact skin limit the permeability of NSAID (Goto *et al.* 1993, Ohara *et al.* 1994).

Multiparticulate dosage forms, such as microspheres (MS), have inherent advantages over conventional dosage forms, as single unit systems, since they spread out more uniformly over absorption sites in the GI tract (Skilson 1985), therefore maximizing the opportunity for drug input as well as minimizing irritation. Several authors have prepared slow release microspheres loaded with ketoprofen and formulated into tablets or capsules (Bianchini *et al.* 1987, El Khodairy *et al.* 1992). In this way, MS prepared with poly-ε-caprolactone (PCL) and cellulose acetate butyrate were proposed for modulating the release of NSAID (Chang *et al.* 1987, Bodmeier *et al.* 1989, Giunchedi *et al.* 1994). Others (Kawashima *et al.* 1993) devised the emulsion solvent diffusion method, by forming an O/W emulsion with acrylic polymers and sugar esters.

Furthermore, the use of pH sensitive polymers, in the form of microparticles, able only to dissolve at pHs around neutrality, inhibits the release of drug from the dosage form in the stomach, avoiding gastric irritation. The main disadvantage in the use of these polymers, with conventional pharmaceutical dosage forms, is the highly variable gastric emptying times, which results in erratic and highly variable blood levels. Liquids and small particles, usually in the micron range, are able to pass through the partially contracted pyloric sphincter into the intestine with the stomach in its fed or fasted mode.

The object of this study has been to obtain and characterize a more efficient and safe ketoprofen dosage form. Different ketoprofen MS formulations based on microparticulate drug delivery systems have been prepared and characterized. MS were made from two water insoluble biodegradable polymers, poly-ε-capro-lactone (PCL) and hydroxypropyl methylcellulose phthalate 50 (HPMCP50), which were used either as pure polymers or mixtures at predetermined PCL/HPMCP50 ratios. These two polymers were selected because of their charac-teristics, so as to achieve a modulation in drug release which would avoid the gastrointestinal adverse effects of ketoprofen as well as improve its bio-availability.

Materials and methods

PCL (50 000 MW) was supplied from Aldrich Chemie (West Germany) and HPMCP50 was obtained from Eastman Fine Chemicals (Quimigranel, Spain). Ketoprofen was from Alkima S.A. (Spain). Polyvinyl alcohol (PVA) was obtained from Fluka (Switzerland). HPLC solvents were purchased from Scharlau (Spain). All other reagents were of analytical grade and were used as received.

Viscosities. The viscosities of HPMCP50 and PCL were evaluated using a LVT Wells-Brookfield microviscosimeter by the method proposed by the USP/NF (USP XXII 1990).

Microspheres. MS were prepared by a modified 'in water' solvent evaporation method (Bodmeier *et al.* 1987). Briefly, a fixed, weighed amount of polymer (1 g) was co-dissolved with variable amounts of drug (100–750 mg) in methylene chloride and emulsified into an aqueous solution of polyvinyl alcohol 0·5% W/V (PVA) during 30 min by means of a stirrer/homogenizer Silverson L2R. Then, the organic solvent was slowly evaporated with gentle magnetic stirring at room temperature and under ambient pressure. The same method was applied to obtain mixed-polymer MS of PCL/HPMCP50 at ratios of 75:25, 50:50 and 25:75.

Drug content. Ketoprofen content in the loaded MS was evaluated in the dried pellets after centrifugation of MS suspensions at 11 000 rpm for 20 min (Biofuge B, Heraeus Sepatech). It is necessary to assess that the drug is not in crystal form (precipitated) in the aqueous phase. In this way, once the MS were prepared, they were observed under optical microscopy. Supernatants were also analysed to ensure that the ketoprofen concentration remained below its maximum aqueous solubility.

Analytical method. The amount of ketoprofen was measured by an external calibrated HPLC modified method (Upton *et al.* 1980). The mobile phase consisted of a mixture 40% MeCN and 60% acetate buffer pH = 3 at a flow rate of 1 ml/min (Waters 510 HPLC pump). The column used was a reversed phase Novapak (Waters RP 18 5 µm, 4·6 × 150) and the UV detector was set at 256·5 nm (Waters 484 tunable absorbance detector). The sensitivity of the method was estimated to be 375 335·10 ± 36 525·19 units of area by mL and µg, and the detection limit was 0·21 µg/mL both at a confidence limit of $p = 0·05$. All the analysis were carried out at ambient temperature (25°C).

Microsphere particle sizes. The sizes of the microsphere particles were determined on a Galai Cis-1 equipment based on the principle of laser light-scattering with a particle size range from 0·5 to 700 µm. Particles of lower size (nanoparticles) present in the samples were determined with a Microtrac ultrafine particle analyser from Leeds and Northrup (range from 5 nm to 2·75 µm).

Scanning electron microscopy (SEM). SEM (Zeiss DSM 950) was used to evaluate the shape and surface characteristics of the prepared MS and MS after incubation in simulated biological fluids. MS were filtered through membrane filters (0·22 µm), dried at room temperature for 24 h and coated in a cathodic evaporator (Polaron) with a gold layer about 50 nm thick.

Size exclusion chromatography (SEC). SEC studies were performed using a PL Gel 10 µm mixed-B 300 × 7·5 mm column (Polymer Laboratories) previously calibrated with polystyrene standards whose molecular weights (MW) ranged from 550 to 5 480 000 Da. The mobile phase was tetrahydrofuran (THF) and tetrabutyl-ammonium bromide at a 1% concentration to avoid polymer associations other than covalent linkage. MS were centrifuged at 11 000 rpm for 20 min, the supernatants containing PVA were discarded, the pellets left to dry and then

dissolved in THF in order to obtain the polymer concentration. SEC studies were also carried out with freeze-dried formulations in order to evaluate the storage conditions.

Differential scanning calorimetry (DSC).   DSC studies of polymer, drug non-loaded and drug loaded MS were made using a Perkin Elmer DSC-2 Differential Scanning Calorimeter. The temperature was calibrated by the melting transition point of indium. Samples weighing about 5 mg were heated at a scanning rate of 10°C/min from 0 to 280°C.

Freeze-drying studies.   Freeze-drying studies were performed as a possible method to improve the stability of MS. Different types of cryoprotectors were tested, glucose, trehalose, mannitol and sorbitol, at two concentration levels 5 and 10% W/V. Also, the freezing rate of samples were evaluated as a variable using liquid N_2 (-196°C) or slow-freezing in a refrigerator at -20°C. For freeze-dried MS, the mean-particle size has been evaluated after reconstitution (S_f) and compared to the initial mean size (S_i) giving a ratio $R = S_f/S_i$ which accounts for the influence of the freeze-drying variables.

In vitro *release experiments.*   Dissolution kinetics from MS were studied in triplicate under non-sink conditions $(C_t = 30\% \, C_s)$. Freeze-dried MS (30 mg) were suspended in 75 ml of dissolution medium in a screw-capped bottle kept at 37°C and stirred magnetically during the experiment. The dissolution mediums used were simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) prepared as described in USP XXII (1990).

Statistical analysis.   All tests were performed by means of a software program (Hintze 1991). The level of significance for the differences found between mean particle sizes was established by t paired tests. The same tests were used to compare the mean values of standard deviations. Drug release from the MS was analysed by one-way ANOVA. The computer performed a Kolmogorov–Smirnov test and a Bartlett test to assure a normal data distribution and the homogeneity of variance.

Results and discussion

Previously we have tested the aqueous solubility of ketoprofen in pure water and in 0·5% PVA aqueous solution, obtaining solubility values of $169·5 \pm 8·1$ μg/mL and $211·5 \pm 3·3$ μg/mL respectively. Also the partition coefficient of ketoprofen between the organic solvent and aqueous surfactant solution was determined (dichloromethane/0·5% PVA aqueous solution: 261). The solvent evaporation method was selected to prepare biodegradable ketoprofen loaded MS from PCL and HPMCP50 considering the low water solubility of the drug and hence low partitioning into the external aqueous phase.

Drug content and incorporation efficiency.   The drug content and incorporation efficiency of the anti-inflammatory agent within polymeric MS, expressed as the percentage of entrapped drug relative to the total amount in each suspension, are shown in Table 1. The encapsulation efficiency decreased by increasing the drug

Table 1.   Encapsulation efficiency (X ± S.D.) of ketoprofen as a function of MS composition.

PCL/HPMCP50 ratio	Theoretical drug content (%)	Actual drug content (%)	Encapsulation efficiency
100:0	7·4	7·25	98·05 ± 1·86
100:0	16·7	13·18	78·94 ± 9·08
100:0	28·5	21·78	76·45 ± 6·82
100:0	37·5	—	drug crystals
75:25	16·7	13·40	80·29 ± 0·70
50:50	16·7	15·96	95·61 ± 1·82
25:75	16·7	16·04	96·05 ± 0·21
0:100	16·7	16·39	98·15 ± 0·77

loading for PCL MS. Statistically significant differences were found between MS with a theoretical drug content of 7·4% versus 16·7 and 28·5%. The highest percentage of drug incorporation (98·15%) was obtained when the MS were made from pure HPMCP50. For polymer blends, the higher the PCL/HPMCP50 ratio the lower the incorporation efficiency. These two polymers display very different solubility characteristics in methylene chloride which was the organic solvent used to obtain the MS. For PCL a clear solution was obtained (viscosity at 20°C is 0·15 poise) while HPMCP50 swells, forming a very viscous gel like structure (viscosity at 20°C > 10·2 poise) but remains insoluble in the organic phase (Florence 1984). To obtain comparable viscosity values the USP method was used. Thus, 10 (w/w) solutions of each polymer in a mixture of 50:50 methylene chloride:methanol (v/v) show a viscosity of 205·60 cps for HPMCP50 and 25·70 cps for PCL. When the MS are forming, the organic solvent diffuses towards the aqueous medium and so does the drug. Thus, the viscosity of the internal phase increases, inhibiting drug partition into the external medium. These two polymers solidify at different rates. While PCL remains dissolved until almost the organic solvent has evaporated, HPMCP50 forms a very viscous dispersion, typical of a swollen polymer, and precipitates as soon as the solvent begins to evaporate because of the polymer desolvation. Drug diffusion is much more facilitated in the first case explaining the lower encapsulation efficiency found for PCL MS.

When PCL was incorporated in HPMCP50 solution, a turbid mixture was obtained suggesting a macromolecular interaction. Thus, the polymers would not be completely mixed and those HPMCP50 nuclei without appropriate solvation would be responsible for larger sizes.

The optical microscope examinations showed the presence of ketoprofen crystals in those PCL formulations where the drug amount was >28·5%. For PCL MS with a theoretical drug content of 16·7 and 28·5, the amount of drug remaining outside the MS exceeded its solubility in the external aqueous medium. However, no crystal was observed under microscopical examination. This fact can be explained by the formation of not only MS but polymeric nanoparticles (NPs) shown by the Tyndall effect displayed by the supernatant. Laser light scattering corroborated the presence of particles of size <1 μm. These NPs are able to entrap lipophilic drugs (Guzmán *et al.* 1990) so they were discarded in the supernatants, thus drug content assays only referred to the ketoprofen encapsulated into the MS. NPs were also observed for PCL/HPMCP50 ratios of 75:25 and 50:50 in which the theoretical drug content was 16·7%.

Table 2. Mean size ± SD (n = 3) for number, area and volume distributions, and mean percent volume particle size distribution for different MS formulations (Theoretical drug content: 16·7%).

|	PCL/HPMCP50 ratio				
	0:100	25:75	50:50	75:25	100:0
	Mean size (μm)				
Number	3.38±0·20	3·56± 0·30	3·30±0·39	4·16±1·04	3·47±0·46
Area	6·01±0·29	12·24± 2·19	11·57±1·88	18·34±8·38	7·08±1·28
Volume	10·94±2·02	37·86± 1·15	30·17±5·13	33·63±6·30	10·77±2·25
	Percentage				
<1 μm (NPs)	0·58±0·45	0·44± 0·23	0·77±0·35	0·59±0·15	0·48±0·13
1–3 μm	3·85±0·46	2·34± 1·78	3·22±0·68	3·14±1·05	5·83±0·86
3–20 μm	88·11±4·01	19·56±11.26	34·47±7·04	34·33±7·27	80·53±7·83
>20 μm	7·46±4·02	77·65±12·93	61·48±7·80	62·05±7·46	13·21±7·24

Particle size. The particle size distribution of the MS, expressed both as particle area and volume showed a marked dependence upon composition (Table 2 summarizes the results). However, there were no statistically significant differences in particle size for the number distributions. In those cases where the MS were prepared from a pure polymer, narrow and very similar particle volume distribution profiles were obtained, $10·94 \pm 2·02$ μm and $10·77 \pm 2·25$ μm for PCL/HPMCP50 0:100 and 100:0 MS respectively ($p > 0·05$). On the contrary, when the MS were made from polymer mixtures, very broad distribution profiles were found. The mean particle size for area and volume distributions showed statistically significant differences ($p < 0·05$) for 25:75, 50:50 and 75:25 PCL/HPMCP50 ratios compared to 100:0 and 0:100 ratios.

Otherwise, for pure polymer MS the volume distribution data indicated that more than 86% of the particles had diameters less than 20 μm, and more than 80% were in the range from 3 to 20 μm. NPs were also detected in all MS formulations samples by Galai-Cis apparatus, but its range of measurement is not suited to analyse particles <0·5 μm. The NPs found in the supernatants were characterized using the Microtrac ultrafine particle analyser. The results suggest that the sizes varied as a function of the polymer ratio. Thus, PCL formulations showed two NP populations of $154·4 \pm 69$ nm (98%) and $684·4 \pm 16·2$ nm (2%). MS made from 75% PCL had only one NP population in the range of $202·8 \pm 90·8$ nm but those from a 50% blend exhibited a complex distribution profile with three populations of $57·3 \pm 13$ nm (26%), $211·7 \pm 78·5$ nm (68%) and $708·9 \pm 170·6$ nm (6%). For MS made from 25% of PCL or pure HPMCP50 monomodal NP distributions were found and the size significantly decreased to $123·5 \pm 54·4$ nm and $117·0 \pm 54·1$ nm respectively. These findings support the hypothesis that polymer interactions can take place, specially when a mixture of 50% PCL/HPMCP50 was used.

Morphological examinations. SEM observation of ketoprofen MS showed spherical particles with different textures and without evidence of crystal formation on the surface. For PCL MS the surface is pitted and has an orange peel-like texture (figure 1a). The MS made from HPMCP50 showed a smooth and non

porous surface (figure 1b). MS sizes estimated from the micrographs was according
to those given by light-scattering. Those prepared from PCL/HPMCP50 blends
were also spherical in shape with very variable sizes compared to the homogeneity
found in PCL and HPMCP50 MS (figure 1c).

Size exclusion chromatography.   Table 3 shows the retention times at the top
of the peaks in the chromatograms obtained for drug loaded MS. Those obtained
for PCL, HPMCP50 and Ketoprofen as raw materials were 7·21, 6·80 and 8·92 min
respectively. These data suggest no interaction between the drug and the polymeric
matrix. No changes were detected during the storage of freeze dried PCL MS.
However, when freeze dried MS made from HPMCP50 or mixtures with PCL
were dissolved in THF and analyzed, three peaks were obtained for all chromato-
grams. Two of them corresponded to the MW of polymer and drug and the last
one with a retention time of 9·60 min remains unknown. To explain this data, other
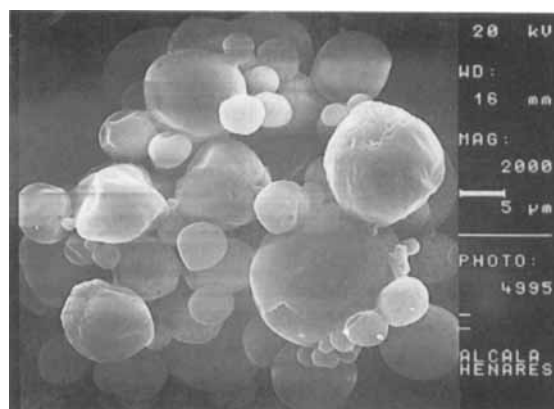

(a)

(b)

M. *Guzmán* et al.
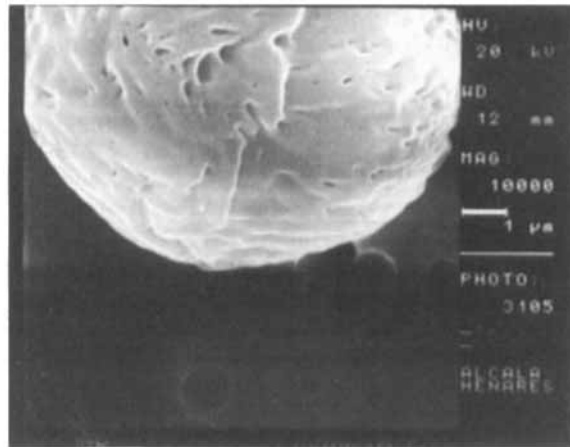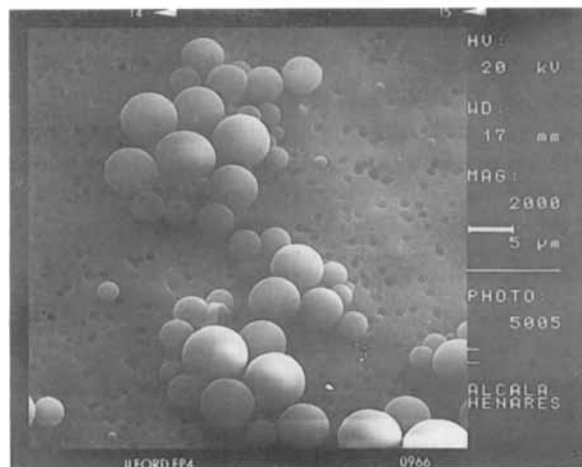
Figure 1. Scanning electron micrographs of microspheres: (a) surface of PCL MS (magnification 10 000); (b) HPMCP50 MS (magnification 2000); and(c) MS made from mixture of 25% HPMCP50 and 75% PCL (magnification 2000).

Table 3. Retention times (min) obtained in SEC chromatograms for PCL, HPMCP50, ketoprofen and different drug loaded formulations of MS, in two experimental conditions: (A) freeze-dried MS dissolved in THF; and (B) Freeze-dried MS reconstituted in water, centrifugated and pellet dissolved in THF.

	A conditions			B conditions	
Ratio PCL/HPMCP50	Polymer	Drug	Unknown	Polymer	Drug
0:100	6·93	9·04	9·58	7·44	8·95
25:75	7·00	9·03	9·60	7·29	8·84
50:50	7·30	8·99	9·55	7·11	8·80
75:25	7·35	9·03	9·60	7·42	8·90
100:0	7·24	8·96	n.d.†	7·27	8·91

† Not detected in this chromatogram.

SEC analyses were carried out. Lyophilizates were reconstituted in water and after centrifugation the supernatant was discarded to evaluate the MW of the dried pellets. The chromatograms obtained did not show the 9·60 min peak suggesting the water solubility of that compound which should have been removed in the supernatant. Furthermore PCL and HPMCP50 MS were assayed with the same protocol and no peaks different from those of raw materials were obtained. This fact points out an apparent degradation of HPMCP50 during MS storage, probably by breaking of the phthalic anhydride bond to the anhydroglucose backbone.

Differential scanning calorimetry. Studies were performed to determine the internal structure of the MS and the physical state of the drug within the carrier. Thermograms were obtained for PCL and HPMCP50 as raw materials, ketoprofen, non-loaded MS and 16·7% drug-loaded MS. Figure 2 shows the thermograms of PCL, ketoprofen and drug loaded PCL MS. A sharp endotherm was observed for ketoprofen at 96·8°C, corresponding to its melting transition temperature. This transition was neither present in drug loaded PCL MS nor HPMCP50 MS
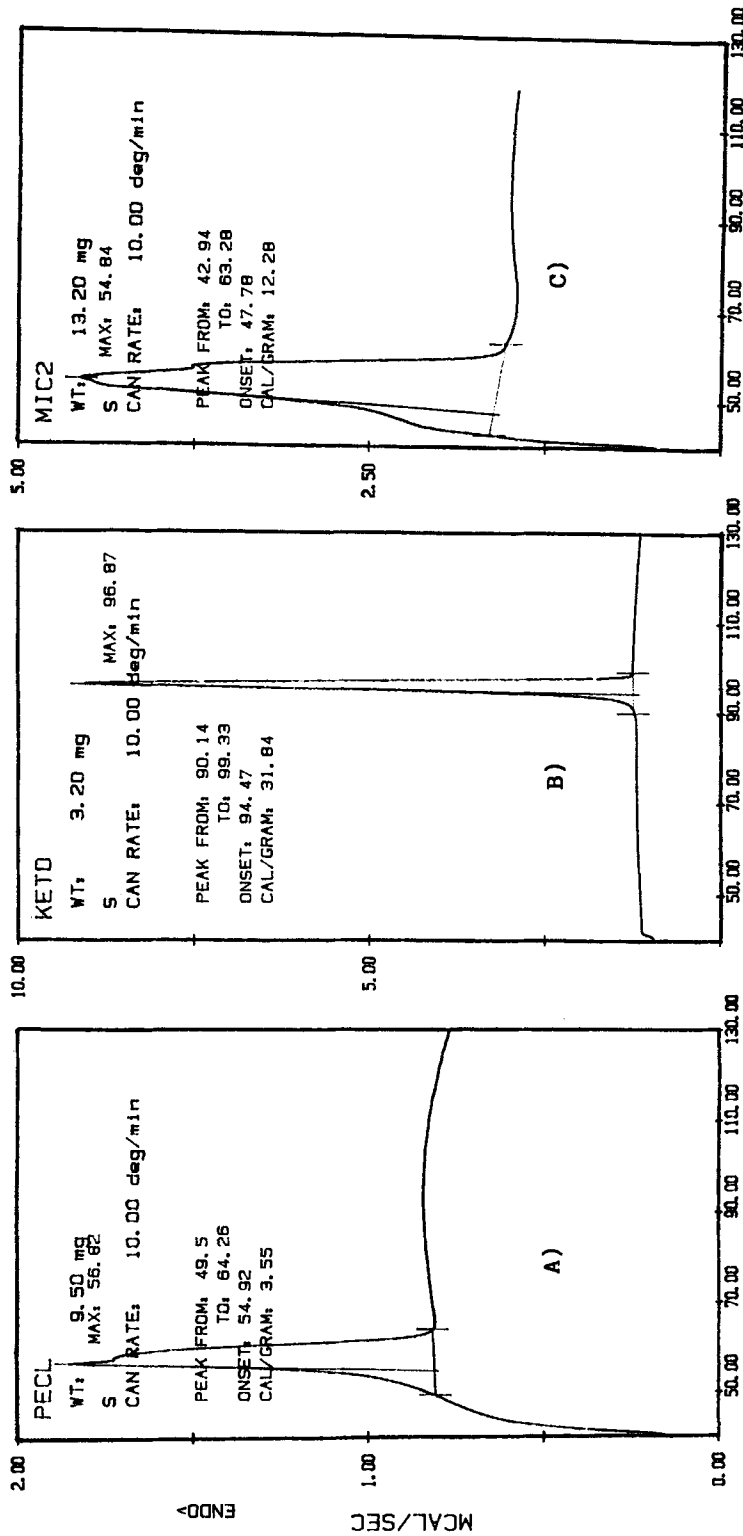
Figure 2. DSC scans of: (a) polycaprolactone; (b) ketoprofen; and (c) drug loaded PCL MS.

thermograms. The melting transition for the crystalline domains of PCL was determined to be 56·8°C. The thermograms of HPMCP50 and HPMCP50 MS showed no endotherms suggesting an amorphous state of the polymer. A slight endothermic change is found at 70°C which could be attributed to the glass transition temperature of HPMCP50 based on the T_g of HPMCP55 given in the literature (97°C). The absence of the drug endotherm in the MS thermograms has been interpreted as drug being either dissolved or molecularly dispersed in the MS. Similar results were obtained by Bodmeier *et al.* (1989). Furthermore, the polymer seems to be more crystalline when processed to form MS since the heat needed to melt 1 g of product increases from 3·55 cal/g for PCL as raw material to 10·88 cal/g for PCL under the form of non-loaded MS. This increase in crystallinity is also observed for drug loaded MS and can be explained by considering a very slow solvent evaporation, allowing the polymer to structure to a greater extent than in manufacturing processes.

Freeze-drying studies.   Stability studies of MS formulations were performed to determine the chemical (drug retention) and physical (particle size) integrity of the carrier as a function of time. A rapid release of the drug from the PCL MS into the external aqueous medium, leading to ketoprofen crystal formation was observed. This fact can be related to the high porosity of the carrier. Based on these findings, another way was needed to achieve the appropriate stability characteristics for MS formulations e.g. to freeze-dry the MS, which is a method already used by many authors, not only for MS, but also for submicron particulate carriers (i.e. nanoparticles) (Auvillain *et al.* 1989). Regarding particle size, figure 3 shows comparatively the quantitative results obtained for PCL and HPMCP50 MS expressed as the relationship (R) between the mean particle size after reconstitution (S_f) and newly-made (S_i) R = (S_f/S_i). Acceptable freeze-drying conditions were those where the differences between S_f and S_i were not >20%. The mean standard deviation of MS populations has also been tested in order to
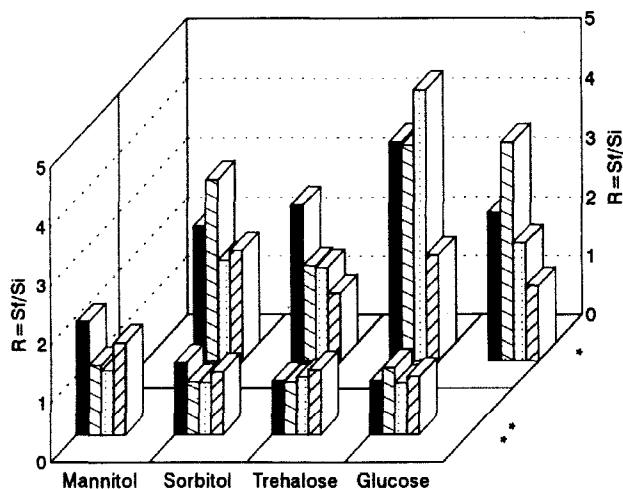

Figure 3.   Effect of type and amount of cryoprotector and freezing temperature on the ratio between sizes of reconstituted and newly made microspheres. Key: (*) PCL MS, (**) HPMCP50 MS, ■5%, −20°C; □10%; −20°C; □ 5%, −196°C; □ 10%, −196°C.

see the similarity or dissimilarity between populations. For HPMCP50 the sizes of MS after reconstitution are nearly the same as initially, except for mannitol at 5% concentration and freezing temperature of $-20°C$ and 10% concentration and freezing in liquid nitrogen. Although sorbitol and mannitol are diastereoisomers, they show different cryoprotection behavior, probably due to polymorphism. Fast freezing conditions led to the formation of a large number of little crystals (LNLC) while a little number of large crystals are obtained under slow freezing. The cryoprotectors are usually employed to induce the formation of LNLC. Mannitol seems to be insufficient to avoid particle agglomeration during the concentration step involved in the freezing process. Furthermore, HPMCP50 is in its glassy state at ambient temperature ($T_g = 70°C$) exhibiting a rigid structure. On the other hand, for PCL MS only, appropriate resuspensions were obtained with 10% of cryoprotector (sorbitol and glucose) at $-196°C$ freezing temperature. This fact is easily explained considering the very low T_g of PCL ($-70°C$). To obtain adequate freeze-dried products it is very important to maintain the sample temperature below its eutectic or glass transition point during primary drying and the cryoprotector do not interact with the substratum. PCL is a semicrystalline polymer with a 50% of amorphous domain which remains in its rubbery state above T_g (Buri *et al.* 1985). Although unsatisfactory, the best results for PCL MS have been obtained by freezing at $-196°C$ (below PCL T_g) which allows the sample to stand during primary drying at a lower temperature than MS frozen at $-20°C$. PCL MS are more porous than HPMCP50 ones thus offering a greater surface area to interact with either water molecules or cryoprotectors. These molecules remaining in the inner structure would freeze at a lower temperature than non-adsorbed ones. During primary drying, adsorbed water can liquefy producing a product collapse. Further studies have to be performed in order to obtain stable PCL MS formulations.

In vitro *ketoprofen release.* Release experiments of PCL/HPMCP50 MS were initially carried out in SGF. This study did not show significant differences ($p > 0·05$) between the polymer mixtures assayed. This fact might be due to the great size dispersion found by light scattering measurements and SEM studies. Based on this results, further *in vitro* release experiments with SGF and SIF were carried out for PCL MS and HPMCP50 MS.

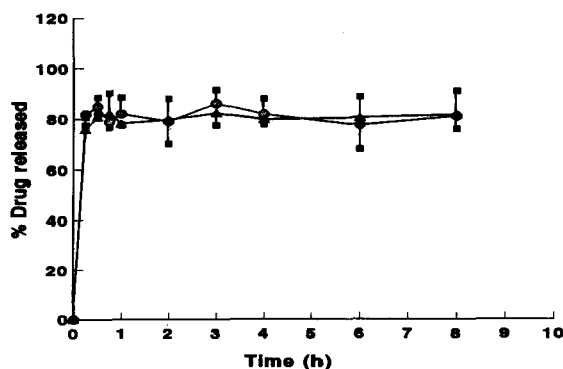*In vitro* release profiles of PCL MS in SGF and SIF are shown in figure 4.

Figure 4. *In vitro* release profiles of PCL microspheres. Key: (●) *simulated intestinal fluid*; (▲) simulated gastric fluid and (■) standard deviations.
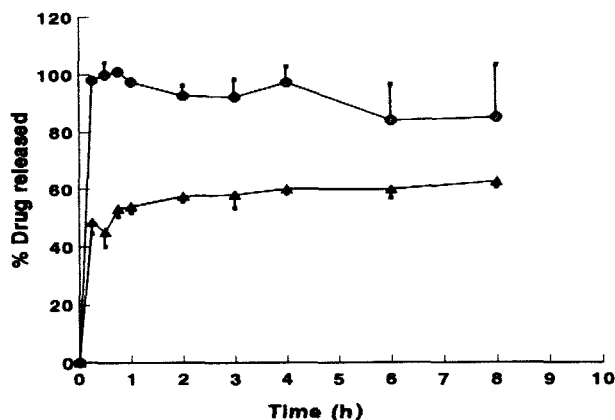
Figure 5. *In vitro* release profiles of HPMCP50 microspheres. Key: (●) simulated intestinal fluid; (▲) simulated gastric fluid and (■) standard deviations.

No significant differences between release profiles in these two mediums were observed. An initial fast ketoprofen release in both cases was found and more than 75% of the drug was released from the MS in the first 15 min. This initial 'burst release' is mainly due to the physical state of the drug in the polymeric matrix and to the porosity of PCL. Other authors have described this 'burst effect' as immediate dissolution of ketoprofen adsorbed on the particle surface (Suchira *et al.* 1992). Figure 5 shows the *in vitro* drug release results in SGF and SIF for HPMCP50 MS. In SGF, ketoprofen release was lower than that observed for PCL MS. The percentage of ketoprofen released from HPMCP50 MS in 15 min and 8 h was about 50 and 65%, respectively, while 8 h release for PCL MS was >80%. *In vitro* drug release for HPMCP50 MS in SIF revealed that more than 90% of the drug was released in the first 15 min. These great differences between ketoprofen release in SGF and SIF from HPMCP50 MS can be explained by the pH influence. The pH value affects ketoprofen solubility but, the pH dependent polymer solubility is the factor that controls drug release from the matrix. In PCL MS, the governing factor controlling drug release in biological simulated fluids is diffusion across the polymeric matrix. During *in vitro* release experiments in SGF and SIF, PCL MS remained intact and no erosion of the polymer was found as shown by SEM (figure 6a). On the contrary, for HPMCP50 MS different behaviour between SGF and SIF was observed. SEM studies showed a maintenance of the particle surface integrity in SGF at 6 h but a significant erosion of polymer matrix in SIF takes place and the particles lost their spherical shape having a foamed aspect (figure 6b). This different erosion is in agreement with the *in vitro* release profiles in SGF and SIF for HPMCP50 MS.

Conclusion

A biodegradable ketoprofen carrier system has been characterized and tested *in vitro*. HPMCP50 MS as well as PCL MS seems able to avoid some of the gastro-intestinal disadvantages of conventional ketoprofen dosage forms and they might improve the dissolution characteristics of the drug, leading to a major absorption and greater bioavailability. When orally administered, HPMCP50 MS
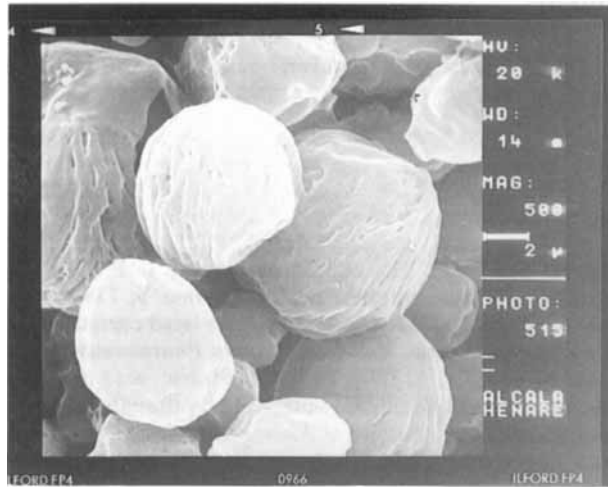
(a)
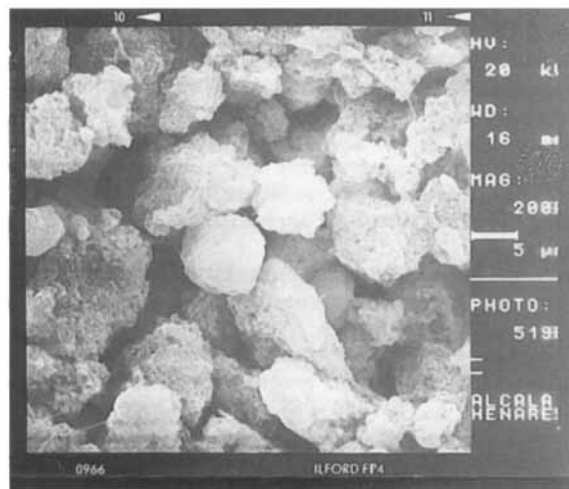
(b)

Figure 6.   Scanning electron micrographs of microspheres after 6 h of *in vitro* release
experiments: (a) PCL MS in simulated gastric fluid (magnification 5000); and
(b) HPMCP50 MS in simulated intestinal fluid (magnification 2000).

would be able to maintain the drug entrapped until the intestinal pH dissolves the
polymer and the release of drug makes possible absorption in the duodenum
thereby minimizing the contact between the drug and the gastric mucosa.
Furthermore, these formulations are not affected by gastric emptying times.
Concerning PCL MS, when administered into a stomach in its fasted mode, the
MS can freely go through the pylorus and reach the intestine without releasing
their content. Once in the intestine MS would be able to be absorbed by different
ways (Kreuter 1991) or adsorbed onto the microvilli and releasing the drug by
diffusion mechanisms. Thus, the contact between the drug and the mucosa would
be avoided.

Acknowledgements

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