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Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly(DL-lactide-co-glycolide) microspheres

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Summary

Empty or nifedipine-loaded biodegradable poly(DL-lactide-co-glycolide) microspheres were prepared by the solvent evaporation method. Spherical microparticles of different size distribution were thus obtained. The evolution of the mean diameter and the extent of size distribution as a function of different preparation parameters were studied. The mean particle diameter decreased when the stirring rate, the surface-active agent concentration and the internal phase volume were increased. Microspheres of 12, 18 and 83 μm, with different nifedipine payloads were obtained. The in vitro release of the drug was evaluated under sink and non-sink conditions: the 12 and 18 μm microspheres provide the same linear profile for approx. 400 h. Under non-sink conditions, the 83 μm microspheres exhibit an S-shape release pattern.

Introduction

Parenteral controlled-release systems are of considerable interest for drugs which either require daily administration, or have high toxicity, or a very low oral bioavailability. Their potential is greatly increased if the dosage form can be injected to the patient. In addition, the use of totally bioresorbable polymers for the realization of these systems avoids the need for surgical residue removal. Microspheres, which consist of a drug dispersed in a spherical polymer matrix, have already extensively been evaluated for the administration of active components such as narcotic antagonists, local anesthesics, steroid hormones, antitumor agents, and several peptides (Beck et al., 1979; Benita et al., 1984; Sanders et al., 1984; Ogawa et al., 1988). The polymer chosen for this study is a lactide-glycolide copolymer which is totally bioresorbable and has excellent biocompatibility (Cutright et al., 1974; Visscher et al., 1985). It is well-known that the physico-chemical characteristics of the polymer and active compounds have a dramatic influence on the

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release rates of the active component from the dosage form (Bodmeier and McGinity, 1987; Mills and Davis, 1987), but it is also important to control all the manufacturing parameters of the delivery system. These parameters determine the properties of the resulting biodegradable product which govern the release patterns of the drug (Wakiyama et al., 1981; Benita et al., 1984). Nifedipine, a calcium-channel blocking agent, practically water-insoluble has been incorporated into poly(DL-lactide-co-glycolide) microspheres by the oil-in-water emulsion-solvent evaporation technique. The aim of this investigation was to study the influence of several preparation parameters (stirring rate, dispersing agent concentration, aqueous and organic phases volumes, nifedipine payloads) on microsphere size distribution, drug contents and nifedipine release profiles.

Materials and Methods

Materials

Poly(DL-lactide-co-glycolide) (PLGA) (Resomer® RG504, Mol. Wt 54 000, lactide/glycolide ratio 50:50) was supplied by Boehringer Ingelheim (Germany). Hydroxypropylmethylcellulose (HPMC) (Pharmacoat 606®) was provided by Seppic (Paris, France) and nifedipine was obtained from Bayer (Leverkusen, Germany). Methylene chloride, methyl alcohol and sodium phosphates, analytical grade, were supplied by Merck (Darmstadt, Germany).

Preparation of the microspheres

PLGA microspheres were prepared according to an emulsion-solvent evaporation procedure (Beck et al., 1979; Arshady, 1991). In order to study the influence of the preparation parameters on the size distribution, samples of 1 g of polymer were dissolved in various volumes of methylene chloride. These organic phases were dispersed under constant stirring in aqueous solutions of HPMC. Stirring was maintained until complete evaporation of the solvent. Microspheres were then washed with deionized water, filtered and dried in a dessicator. For emulsification of the organic polymer solution, an Ika RW18 stirring apparatus (Janke & Kunke, Ika-Werk, Staufen, Germany) equipped with a 52 mm diameter propeller was used. For the studies with an active component entrapped in the polymer matrix, nifedipine was co-dissolved with the polymer in the organic phase. As nifedipine is a very photosensitive molecule, all the preparation steps were carried out in a dark room.

Size distribution determination

The mean diameter and the particle size distribution of the different batches were determined with a Coulter counter model A (Coulter Electronics Ltd, Luton, U.K.) equipped with a 140 or 200 μm aperture diameter tube. Large microsphere batches were sized by optical microscopy.

Viscosity measurements

The viscosity of the organic phase was evaluated at 20°C with a capillary viscosimeter (Ubbelhode). Several PLGA concentrations in methylene chloride were analysed.

Evaluation of nifedipine contents

A known amount of nifedipine-loaded microspheres was dissolved in a small volume (2 ml) of methylene chloride. Methyl alcohol was then added to a total volume of 10 or 25 ml, depending on the amount of nifedipine present, in order to precipitate the polymer which was then centrifugated. Appropriate dilution of the supernatant was carried out before the solution was injected in a HPLC system. All nifedipine handlings were carried out in a dark room, and flasks were wrapped in aluminium foil.

The HPLC system consisted of a Waters 6000A solvent delivery pump (Waters, Milford, U.S.A.), and a UV detector (Pye-Unicam). The detection wavelength was set at 236 nm. Separation was achieved by using a reversed phase column (Chromspher C18 from Chrompack Belgium N.V., Antwerpen, Belgium), and a flow rate of the mobile phase of 1.0 ml/min. The mobile phase consisted of methyl alcohol/0.01 M aqueous phosphate buffer pH 6.1 (45:55).

In vitro dissolution studies

Samples of approx. 10 mg of microspheres were placed in flasks containing 250 ml of 0.1 M

phosphate buffer pH 7.0. The flasks were immersed in a water shaker bath maintained at 37°C in complete darkness. Aliquots were withdrawn at successive time intervals, and the nifedipine concentration was evaluated after injection in the HPLC system described above. The dissolution medium was maintained at a constant volume by adding a volume of fresh buffer after each withdrawal.

It is important to note that for practical reasons, these tests were not performed under sink conditions. The very poor aqueous solubility of nifedipine (about 10 μg/ml) did not permit sink conditions to be maintained because volumes of release medium of more than 1 l would have been involved. In order to enable the use of such volumes, a flow through cell method was developed. For this technique, 2.5 l of pH 7.0 phosphate buffer was allowed to run through a special cell for powders (Dissotest CE6, Sotax, Basel, Switzerland) maintained at 37°C and containing 10 mg of microspheres. The flow rate of the buffer in the closed circuit was set at 8 ml/min.

Results and Discussion

Several preparation parameters of the microspheres were changed in order to evaluate their influence on the resulting product: the surface-active concentration in the aqueous phase, volume of the aqueous phase, volume of the organic phase, stirring rate, and nifedipine loading. All batches of microspheres were prepared at least in duplicate. In a first step, drug-free microspheres were prepared in order to determine the exact influence of manufacturing conditions on their size distributions. In the second step, various amounts of nifedipine were added during the preparation process, and the influence of the nifedipine contents on the mean diameter was observed. In the same way, the release of nifedipine from microspheres was determined in order to study the influence of drug contents and particle size on the release profile.

Influence of the amount of the dispersing agent and of the stirring rate

In preliminary experiments, it was observed that for very low amounts of HPMC, spherical microparticles were obtained, but with a larger diameter than for higher levels. Moreover, many polymer aggregates were formed during the manufacturing process. In order to eliminate these aggregates, the minimum HPMC concentration used for this study, was set at 0.4% (w/w). Different concentrations of HPMC in the aqueous phase were tested, from 0.4 to 2.4% (w/w), and the stirring rate was varied between 250 and 1600 rpm. For the lower HPMC concentrations (0.4 and 0.8%), the particle size distributions were large (8–80 μm) and irregular whereas at higher dispersing agent concentrations, smaller microspheres with narrow size distributions (4–48 μm) were obtained (Table 1).

Similarly, Table 2 shows that when the stirring rate was increased, the microparticles became smaller and the size distribution narrow. Both dispersing agent and stirring rate are parameters of primary importance in the emulsification steps. The stirring rate provides the energy, and the surface-active agent decreases the interfacial tension between the organic droplets and the aqueous medium. For high levels of HPMC concentrations and stirring rates, energetic conditions are appropriate for the maximum division of the organic phase, so that small mean particle diameter and narrow particle size distribution are obtained.

Influence of the aqueous phase volume

The volume of the aqueous phase was changed between 100 and 500 ml without varying the

TABLE 1

Influence of several amounts of the dispersing agent on the mean diameter of the microspheres

HPMC concentration (%)	Mean diameter (μm)	S.D. (μm)	Relative error (%)
0.4	28.5	15.4	0.7
0.8	23.5	13.8	1
1.2	21.5	11.0	6
1.6	19.9	10.2	3
2.0	16.5	8.8	3
2.4	12.9	6.6	2

Other manufacturing parameters were maintained constant: stirring rate, 800 rpm; organic phase volume, 20 ml; aqueous phase volume, 250 ml ($n \geq 2$).

TABLE 2

Influence of the stirring rate on the size of the microspheres

Stirring rate (rpm)	Mean diameter (μm)	S.D. (μm)	Relative error (%)
250	38.1	18.2	10
400	23.4	12.9	8
600	20.5	11.7	7
800	19.9	10.2	4
1200	15.6	8.4	1
1600	13.7	6.6	8

HPMC concentration, 1.6% w/w in 250 ml of water; methylene chloride, 20 ml ($n \geq 2$).

geometry of the manufacturing system (same beaker and stirrer). No evolution of the size of microparticles was observed with the different volumes used, as shown in Table 3. It may be concluded that this volume does not influence the formation of the microspheres within the limits of our preparation conditions.

Influence of the organic phase volume

Different volumes of methylene chloride were emulsified in the aqueous phase, without changing the amounts of polymer or emulsifier. It was observed that variations of this volume have a strong influence on the size of the microparticles: the smaller the organic phase volume, the larger the mean diameter with a wider size distribution. The decrease in the mean diameter as a function of the increase in organic phase volume is shown

TABLE 3

Variation of the size of the microspheres as a function of the aqueous phase volume

Aqueous phase volume (ml)	Mean diameter (μm)	S.D. (μm)	Relative error (%)
100	19.5	10.1	1
175	18.7	10.6	10
250	19.9	10.2	3
350	18.5	9.4	8
500	16.4	8.9	–

Organic phase volume, 20 ml; HPMC concentration, 1.6% (w/w); stirring rate, 800 rpm.

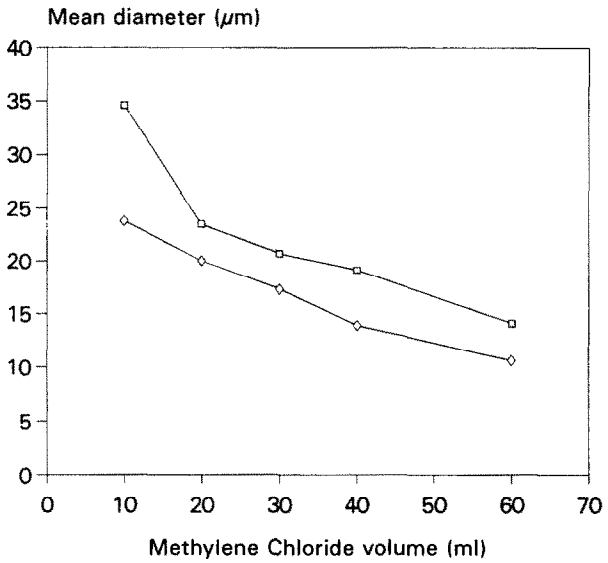

Fig. 1. Influence of the organic phase volume on the mean diameter. The experiment was performed at two different HPMC concentrations ((□) 0.8 and (◇) 1.6% (w/w)), the stirring rate being 800 rpm and the aqueous phase volume 250 ml ($n \geq 2$).

in Fig. 1. These results can be explained on the basis of the viscosity of the internal phase of the emulsion increasing with decreasing volume, the amount of polymer being kept constant. Table 6 shows the influence of the PLGA concentration in methylene chloride on the viscosity of the organic phase.

Size and nifedipine contents of microspheres

Among the different manufacturing parameters tested without any drug, different methods were chosen to evaluate the incorporation of nifedipine: the first involved a small volume of methylene chloride (20 ml) in order to provide microspheres of about 20 μm, the second being carried out with 60 ml of organic phase and the mean diameter obtained was around 10 μm. All the other parameters were maintained constant: 1.6% (w/w) HPMC, 800 rpm and 250 ml of aqueous phase. Both methods were chosen since they lead to the formation of microspheres with more readily reproducible and regular size distribution than other methods which provide larger microspheres. A third method was developed in order to obtain larger microspheres: the amount

TABLE 4

Mean diameter and mean drug contents of several nifedipine-loaded microsphere preparations

Nifedipine amount (mg)	Methylene chloride (ml)	HPMC amount (%)	Stirring rate (rpm)	Mean diameter (μm)	Nifedipine contents (% w/w)	Yield of encapsulation (%)
0	20	1.6	800	19.9	0	–
0	60	1.6	800	11	0	–
80	20	1.6	800	16.7	6.8	92
80	60	1.6	800	10.3	7.4	100
200	20	1.6	800	18.0	14.4	86
200	60	1.6	800	10	14.9	90
200	20	0.8	250	80.5	13.6	82
250	20	1.6	800	20.2	19.0	95
250	60	1.6	800	9.7	18.8	94
300	20	1.6	800	17.8	22.7	99
300	60	1.6	800	11	23	101

The amount of polymer was maintained constant (1000 mg) ($n \geq 2$).

of HPMC and the stirring rate were reduced to 0.8% (w/w) and 250 rpm, respectively. In order to reduce the extent of the size distribution and to eliminate the small microspheres and aggregates, the particles were sieved between 80 and 160 μm.

The mean sizes and drug contents of microspheres prepared by using four loading levels of nifedipine are listed in Table 4, and compared with those of the drug-free microspheres. The mean yields of nifedipine incorporated into the microparticles are very high (80–100%) as nifedipine is a very poor water-soluble drug. Therefore, excellent manufacturing conditions were established for the maximum incorporation of this drug which underwent preferential partitioning into the organic phase of the emulsion. In such a situation, very low amounts of drug were lost in the aqueous phase. It can be observed that the mean particle diameter is not influenced by the nifedipine contents of the microspheres.

Good reproducibility in the preparation of microspheres is achieved by using the same experimental procedure and by carefully maintaining

TABLE 5

Linear release between 24 and 350 h

Microsphere characteristics		Linear regression: $y = ax + b$		
Mean diameter (μm)	% nifedipine	a (% released/h)	b (% released)	r
Non-sink conditions				
11.7	14.7	0.238	4.9	0.982
11.7	22.5	0.215	14.4	0.991
16.8	6.5	0.171	16.3	0.984
17.5	13.3	0.148	15.7	0.963
18.8	22.8	0.167	18.9	0.989
Sink conditions				
11.7	22.5	0.188	17.8	0.983
18.8	22.8	0.191	13.4	0.978

TABLE 6

Viscosity of the organic phase for different PLGA concentrations in methylene chloride

CH_2Cl_2 volume (ml)	PLGA amount (g)	PLGA concentration (% w/v)	Organic phase viscosity (20°C) (mPa s)
10	1	10	79.6
20	1	5	25.6
40	1	2.5	12.5
60	1	1.7	9.6

conditions such as stirring rate, surface-active agent concentration, and organic phase volume constant.

It should be borne in mind that no free drug crystals appeared when microspheres were prepared according to the above-described conditions. In contrast, when the amount of nifedipine was increased above 300 mg, free crystals were formed. Thus, the maximum nifedipine content used in this study was around 22%.

In vitro nifedipine release

In the initial experiments, the release tests were performed under non-sink conditions. Several preparations characterized by different mean particle sizes and drug loadings were evaluated: two batches of 12 μm microspheres having drug contents of 14.7 and 22.5%, respectively, and three batches of 18 μm microspheres with drug contents of 6.5, 13.3 and 22.8%, respectively. These batches were especially chosen because they are characterized by reproducible and regular size distribution of the particles. Another batch of which the size had been controlled by sieving was selected: 83 μm and 15.1% nifedipine. In their release pattern, the batches of 12 and 18 μm exhibited an initial burst effect (approx. 10% released after 5 h) (Figs 2 and 3), which was absent for the 83 μm microspheres (Fig. 4). This was attributed to the immediate dissolution of the portion of nifedipine located at the surface of the microspheres. In the case of the smaller size batches, the surface area is very large and hence the amount of drug is considerable and can provide massive and rapid release. In contrast, the larger microspheres have a smaller surface and

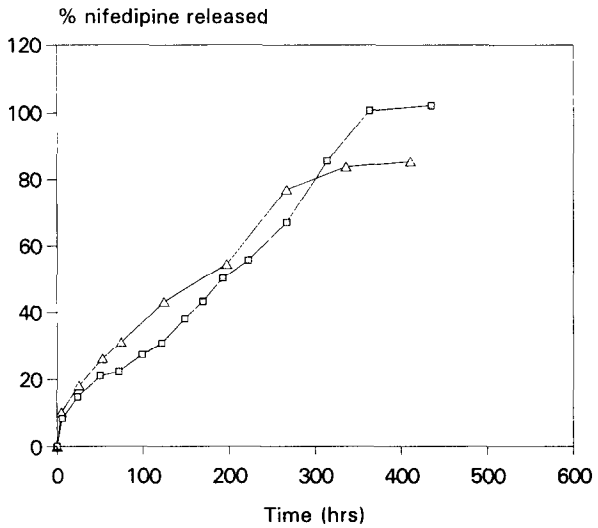

Fig. 2. Release profiles of 12 μm microspheres containing (□) 14.7 and (△) 22.5% nifedipine.

no burst effect can be observed. Between 12 and 18 μm, the burst appears to be influenced by neither the size of the microspheres nor the nifedipine contents. Both sizes are probably too close to allow the detection of any difference. On the other hand, the microspheres loaded with 6–22% of nifedipine are characterized by the same burst effect, which indicates that the percentage of nifedipine located on the microsphere

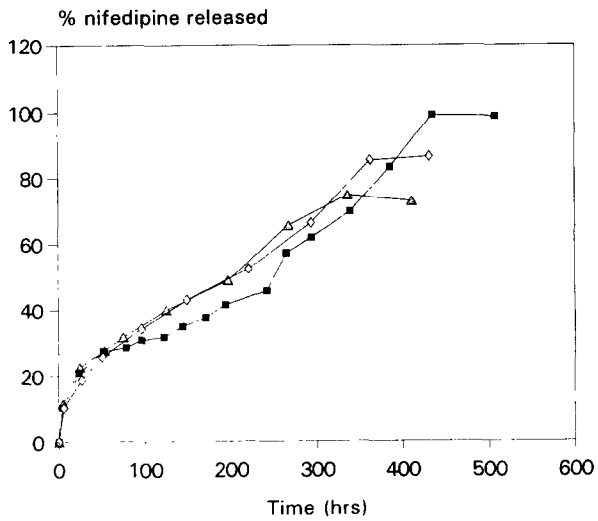

Fig. 3. Release profiles of 18 μm microspheres loaded with (◇) 6.5, (■) 13.3 and (△) 22.8% nifedipine.

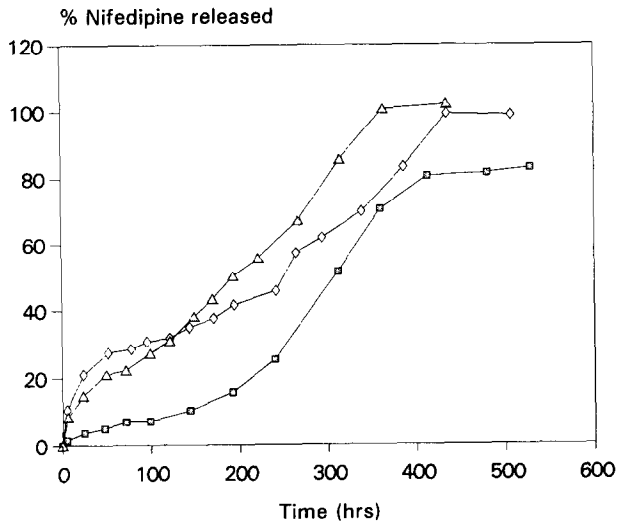

Fig. 4. Release profiles of microspheres loaded with 14% nifedipine as a function of particle size: (△) 11.7, (◇) 17.5 and (□) 83 μm.

surface remains constant when the total amount of the active compound increases from 6 to 22%.

After the burst, the 12 and 18 μm batches exhibited practically linear release profiles between 24 and 350 h (Table 5).

The 12 and 18 μm batches released about 90–100% of their total drug contents within approx. 400 h, except the microspheres with payloads of 22% which showed a steady-state at around 70% release. The larger microspheres (83 μm) exhibited an S-shaped release pattern: an initial lag time of 150 h followed by linear release and a steady state at 80% after 400 h. The first very slow release period can be attributed to a small surface, slower penetration of water into the polymer matrix, and finally to a greater distance for the diffusion of the active substance.

The 70% steady state for the batches with higher load could be attributed to the non-sink conditions. Therefore, a flow through cell method was developed in order to determine the release profile of nifedipine from microspheres under sink conditions. A total volume of 2500 ml was used in a closed circuit. Only batches with the highest drug contents were tested this way. Figs 5 and 6 compare the release under sink and non-sink conditions for 12 or 18 μm microspheres containing about 22% nifedipine. Microspheres

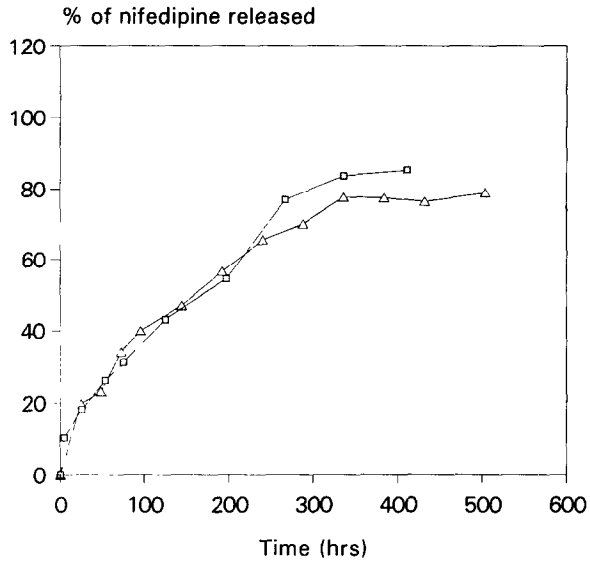

Fig. 5. Sink (2500 ml, △) and non-sink (250 ml, □) release profiles of 12 μm microsphere loaded with 22.5% nifedipine.

of 83 μm were not tested under sink conditions because the preparation parameters chosen to obtain this size did not allow drug contents of 22% to be attained.

No real difference can be observed in the release patterns obtained under sink and non-sink

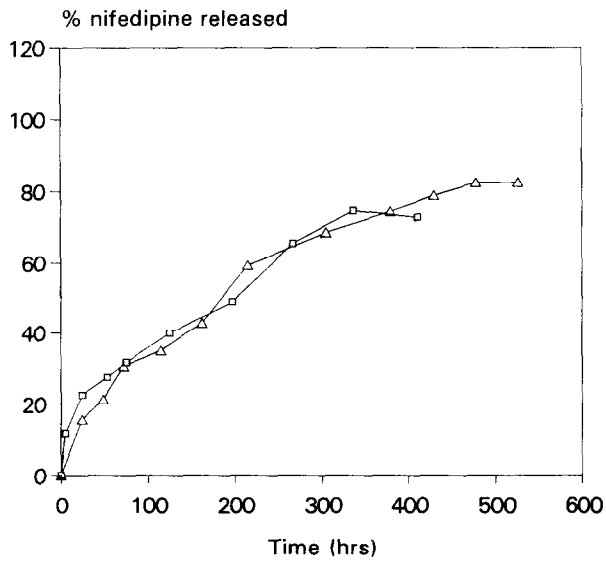

Fig. 6. Flask (250 ml, □) and flow through cell (2500 ml, △) release profiles of 18 μm microspheres loaded with 22.8% nifedipine.

conditions. The slopes of the curves obtained with the flow through method, between 24 and 350 h, are very similar to those determined under non-sink conditions. At this stage of our work, this phenomenon can only be explained on the basis of the slow release from the polymer being the real limiting step of the release process.

Conclusion

This study has shown that the control of some manufacturing parameters for poly(DL-lactide-co-glycolide) microspheres, such as the emulsifier concentration (HPMC), stirring rate and organic phase volume, is of primary importance in order to obtain batches of microspheres of reproducible sizes. The incorporation of nifedipine can be achieved with very high yields. The release of nifedipine from microspheres of three different sizes and with several drug loadings occurs in around 400 h, under both sink and non-sink conditions. The release patterns obtained for the 12 and 18 μm batches are linear after a burst effect, while the 83 μm microspheres display an S-shape profile. In the same way, the two tested methods did not allow us to observe any difference in the release of nifedipine from microspheres with different drug loadings.

The microspheres obtained under these manufacturing conditions, having narrow size distributions and relatively small mean diameters between 12 and 18 μm, provide a constant in vitro release of nifedipine between 24 and 350 h. Their in vivo behaviour should be studied in order to detect eventually any differences in the release patterns. Further investigations are being undertaken including the evaluation of the rate of biodegradation of the microspheres and the study of the internal structure of the matrix to explain the in vitro release profiles obtained.

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