



Methadone implants for methadone maintenance treatment. In vitro and in vivo animal studies

C.M. Negrín, A. Delgado, M. Llabrés, C. Évora*

*Departamento de Ingeniería Química y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de La Laguna,
38200 La Laguna, Spain*

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Abstract

Methadone implant formulations elaborated with polylactide-co-glycolide (PLGA) and polylactic acid (PLA) for 1 week and 1 month release duration, respectively, were evaluated in vitro and in vivo. One-week implants prepared with methadone clorhydrate, methadone clorhydrate/methadone base blend or methadone base were tested in vitro. Results showed that the methadone release rate decreased as the methadone base increased. The best release profile was achieved when the methadone base implants, made by compression of a 50:50 PLGA (12 kDa) and methadone base mix, were coated with PLA (30 kDa). For 1-month implants, the methadone base load was increased to 65% and PLA of 30 kDa was used as a matrix component. In this case the implants were coated with the same polymer. Deconvolution methods could not be used for in vivo release estimation because an increase in methadone clearance was observed with methadone clorhydrate solution multiple-dose treatment. Therefore the amount of drug remaining within the implants was evaluated and the deconvolution was only used to establish the release profile range. The upper limit was estimated applying the absorption–disposition function obtained after multiple-dose administrations while the lower curve was estimated using the single-dose function. Methadone serum levels were maintained around 200 ng/ml during 1 week and approximately 5 weeks with the optimised implants. In vivo–in vitro correlations were always very good with slopes near 1.

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1. Introduction

Methadone has been widely used for opiate addiction for rehabilitation and social adjustment of chronic heroin users. The main reasons are due to its properties of effective suppression of opiate with-

drawal symptoms for long periods, oral absorption, long duration of action and slow excretion. Methadone maintenance treatments (MMT) are designed for heroin addicts in a similar manner to that of therapy for other chronic illnesses [1]. However, these programs require daily oral administration and, consequently, regular visits to dispensaries, which in most cases results in a lack of patient compliance. Some long-term drug abusers respond well when methadone treatment is terminated but the majority experience a return of symptoms, so recid-

* Corresponding author. Tel.: +34-922318507; fax: +34-922318506.

E-mail address: cevora@ull.es (C. Évora).

ivism is a life-long risk. Approximately 70% of the patients may relapse and criminal activities or needle sharing may re-occur [2].

The use of a controlled release formulation for a long period of time could be the best way to improve the efficacy of the treatment, patient compliance and social rehabilitation. Little attention, however, has been paid to this type of formulation for methadone. A few papers can be found in the literature about *in vitro* release studies such as those performed by Cha and Pitt [3] and Delgado et al. [4] using biodegradable microspheres that release 70–80% of the drug in approximately 1 week.

One reason for the lack of attention to these kind of formulations could be the wide range of therapeutic concentration, from 50 to 600 ng/ml, found effective for MMT [5]. However, according to Eap et al. [6], most of the studies have been performed only on a small number of patients. From this point of view, the results obtained by these authors using a larger number of patients seem more reliable, supporting the existence of different L-methadone thresholds of 200, 250 and 300 ng/ml, although the most effective results were found at 250 ng/ml.

In previous works our team studied the design and *in vitro*–*in vivo* optimization of DL-poly(lactic acid) microspheres containing DL-methadone for delivering in a week [4,7]. However, the methadone serum levels obtained after microsphere subcutaneous administration were much lower than those expected from the *in vitro* release results, probably due to a low bioavailability from the microspheres together with a metabolic auto-induction process [8,9].

The aim of this work was to develop and optimise a controlled delivery implant for long-term methadone maintenance treatment, bearing in mind that methadone levels must be kept in the therapeutic range during the time the release lasted and that an auto-induction process could take place. The first step was to study the methadone pharmacokinetics in mice after single- and multiple-dose regimens to establish the dose range. Then implants for 1 week and 1 month release were developed and tested in mice through the methadone levels in serum.

2. Materials and methods

2.1. Materials

Methadone clorhydrate was supplied by Alcaliber (Spain). Methadone base was obtained from the clorhydrate by precipitation with 1 N NaOH. The implants were made using poly(DL-lactic acid) (Resomer® R104, Resomer® R203, Resomer® R207) and poly(D,L-lactide-co-glycolide) (Resomer® RG502) which were purchased from Boehringer Ingelheim, Germany. All other chemicals and solvents were reagent grade.

The average molecular weights (Mw, Mn) and polydispersity (pd) of the different polymers used to prepare the implants (Table 1) were determined, as previously described [10], by gel permeation chromatography (GPC).

2.2. Implant preparation

The implants were prepared by compression of a mixture of methadone (base or clorhydrate) and PLA or PLGA in different ratios using a Carver hydraulic press at 433 or 347 MPa for 5 min at room temperature. The 347 MPa implants (tablets) were prepared by compressing 26, 48 or 70 mg using a 6-mm punch, while 500 mg of the mix was compressed with a 12-mm punch to make the high-pressure implants. The 12 × 3 mm tablets were divided into smaller parallelepipeds (slabs) 3 × 2 × 1 mm. An empty mold of approximately 3 × 2 × 1 mm was used as a model to keep the size uniform.

Some implant lots were coated with a solution of PLGA or PLA in methylene chloride. This coating solution was applied on the implants' surface using a brush and set aside at room temperature and atmospheric pressure until completely dried. The coating thickness was determined by measuring the implant

Table 1
Average molecular weights and polydispersity determined by GPC of the polymers used in the implant elaboration

Trade name	Mw (kDa)	Mn (kDa)	pd	Named as
Resomer® R104	5.9	4.7	1.25	PLA-5.9
Resomer® R203	28.6	18.7	1.53	PLA-30
Resomer® R207	231.6	135.1	1.71	PLA-230
Resomer® RG502	12.9	8.5	1.51	PLGA-12

thickness before and after the coating process (Micromeritics, Mitutoyo, Japan). The reproducibility of the coating method was tested through the *in vitro* release profile using the similarity factor (f_2).

Methadone content was determined, in triplicate, by spectrophotometry at 290 nm dissolving the implants in methylene chloride.

2.3. *In vitro* release assay

Release of methadone from the implants was assayed in triplicate under sink conditions. The implants were placed in flasks with pH 7.4 isotonic phosphate buffer solution (37 °C). At suitable time intervals, an aliquot of the aqueous solution was withdrawn and replaced immediately with fresh buffer. Methadone release was assayed by spectrophotometry at 207 nm in acid medium.

2.4. *In vivo* experiments

The Committee on Animal Experimentation of University of La Laguna had previously approved the protocol of the study.

The experiments were carried out in a total number of 276 male Swiss mice (28–32 g) purchased from the University Central Animal House. The animals were kept at a controlled temperature of 20 °C and a humidity level of 70% with a normal 12-h light/dark cycle starting 5 days before treatment and during it. The animals had free access to food and water all the time.

2.4.1. Administration protocols

Single- and multiple-dose protocols were followed to determine the pharmacokinetics of methadone in mice:

- (a) Single-dose protocol: a solution of methadone clorhydrate, corresponding to 6 or 12 mg/kg of methadone base, in 50 μ l of saline solution was injected subcutaneously between the shoulder blades of the mice.
- (b) Multiple-dose protocol: a solution of methadone clorhydrate equivalent to 12 mg/kg of methadone base was injected subcutaneously at 12-h intervals over 4 days. After a 15-h wash period ($>10 t_{1/2}$), a new injection of 12 or 6 mg/kg was administered.

Implant protocol: The implants were surgically placed in the back of mice anaesthetised with ether.

2.4.2. Sampling schedule and methadone analysis

A group of four mice were sacrificed and samples for methadone analysis were collected for each time point sampled.

(a) Blood samples were obtained by cardiac puncture:

- Pharmacokinetic studies: samples were collected at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h.
- Implant studies: samples were collected during 1 week at 4 h and each day during 6 or 7 days depending on the formulation under study. For 1-month implant, samples were collected at 4 h and then at 1, 3, 7, 10, 14, 17, 21, 24, 28, 35 and 42 days.

The serum were separated by centrifugation and stored at -80 °C for TDX analysis. The specific modification proposed by Dessalles et al. [11] was used to adapt the TDX kit (ABBOT®) for urinary methadone assay to the serum determination. A TDX calibration curve was prepared for each new reagent kit. The polarization error (PERR) was between -1.93 and $+1.96$ for all the calibrators used (50, 100, 200, 400 and 800 ng/ml) and the root mean squared error (RMSE) was between 0.07 and 0.95. A methadone control level was also run for each sample carousel. The precision level was determined by assaying four replicates each of methadone in mouse serum at 50, 100 and 200 ng/ml. The coefficients of variation (C.V.) obtained were less than 4.5%.

(b) Implant samples: The implants were removed free of tissue from the back of the four mice at the same point in time that the blood was sampled. They were then dissolved in methylene chloride for the remaining methadone determination by spectrophotometry as described above (Section 2.2).

2.5. Pharmacokinetic analysis

Serum concentration of methadone versus time profiles were fitted to an open bi-compartmental model. The input function for the sustained release system includes two processes: the release kinetics and a first-order absorption.

Parameters of the poly-exponential characteristic function were estimated by nonlinear regression using the *Mathematica* function NonlinearRegress [12].

The amount of methadone delivered in vivo from the system was calculated by direct deconvolution method [13,14] using the symbolic computation of the *Mathematica* program [12].

3. Results and discussion

3.1. Pharmacokinetic analysis

The implant dose for reaching therapeutic levels was established by estimating the methadone serum clearance (Cl_s). After 6 and 12 mg/kg doses, the estimated Cl_s were 10 and 7.4 l/h/kg, respectively, significantly different ($p < 0.05$) and higher than the values of 6 l/h/kg given for rats [15], 0.186 l/h/kg for opioid addicts and 0.093 l/h/kg for chronic pain patients [16,17]. However, for the multiple-dose regimens, values of 13.9 and 13.7 l/h/kg were estimated for 6 and 12 mg/kg doses, respectively. These values are significantly higher ($p < 0.05$) than those obtained from a single dose, showing that auto-induction of the methadone metabolism took place with the repeated doses (Fig. 1).

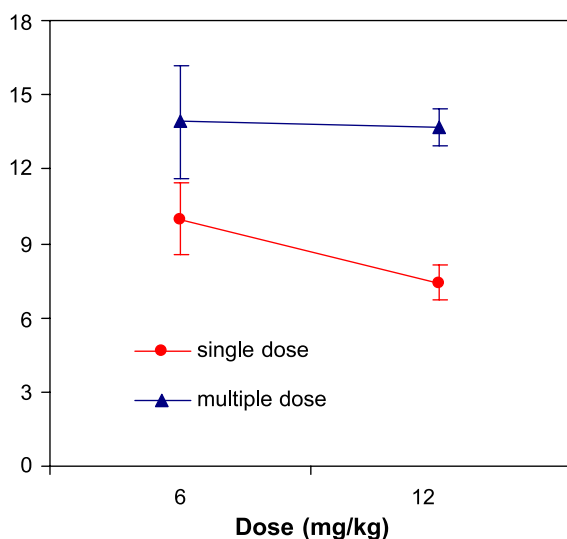


Fig. 1. Estimated methadone serum clearances following single- and multiple-dose protocols after administration of 6 and 12 mg/kg of methadone.

Given that the methadone therapeutic levels are not well established, we have taken as reference the 100–200 ng/ml values given by Cha and Pitt [3] since, to our knowledge, they are the authors who have also been trying to obtain a sustained release formulation for methadone. We have also taken into account the 200–300 ng/ml range suggested by Eap et al. [6]. The appropriate methadone dose needed to achieve therapeutic levels in serum between 100 and 200 ng/ml ranges from 0.5 to 2 mg/day and was calculated by using the limits of the Cl_s range for mice of 30 g (0.22–0.42 l/h).

3.2. One-week implants

Three groups of 1-week implants were made: methadone clorhydrate implants, methadone clorhydrate/methadone base blends implants and methadone base implants. The implant characteristics are shown in Table 2.

3.2.1. Methadone clorhydrate implants

The methadone clorhydrate implants, lot 1, were of slab form (Table 2). The in vitro release profile presented a high burst effect and most of the drug was released in 24 h. Consequently, the implants were coated with PLGA-12 (12 kDa), PLA-5.9 (5.9 kDa) and PLA-230 (230 kDa) at 12% in methylene chloride. The coating thickness obtained is given in Table 3. The coating reduced the release rate but the percent released during the first 24 h was still too high, ranging between 50% with PLA coatings and 70% with the PLGA one.

3.2.2. Methadone clorhydrate/base blends implants

Since methadone clorhydrate is much more soluble than the base, methadone base was introduced in the formulation to reduce the release rate. Two lots were made, lot 2 (Table 2) with 25:25 of clorhydrate/base and maintaining the 50% of PLGA-12 and lot 3 (Table 2) reducing the polymer to 25% in order to introduce 25% of methadone base. Both implant lots were coated with the same polymers (PLGA-12, PLA-5.9 and PLA-230) at the same concentration in methylene chloride (12%) (Table 3). The presence of methadone base reduced the release rate and the effect of the coating was more intense on lot 3 because of the higher methadone clorhydrate proportion.

Table 2
Characteristics of the different implant lots

Lot	Polymer (%)	Methadone (%)		Shape	Weight (%)	Loading (%)
		M_{HCl}	M_{base}			
1	PLGA-12 (50%)	50	–	slab	10.1 ± 0.1	50.4 ± 0.60
2	PLGA-12 (50%)	25	25	slab	9.85 ± 0.6	50.8 ± 0.45
3	PLGA-12 (25%)	50	25	slab	10.1 ± 0.6	49.3 ± 1.40
4	PLGA-12 (50%)	–	50	slab	10.0 ± 0.2	50.3 ± 0.27
5	PLGA-12 (50%)	–	50	tablet	26.2 ± 0.5	49.3 ± 1.59
6	PLA-30 (35%)	–	65	tablet	48.0 ± 0.3	64.5 ± 0.90
7	PLA-30 (35%)	–	65	tablet	70.2 ± 0.6	65.1 ± 1.1

Reproducibility of the coated implants release profile was good for clorhydrate and clorhydrate/base blends implants, obtaining similarity factor (f_2) values >50.

The in vitro release results obtained with both types of implants showed that a reduction of the initial release rate can be reached increasing the methadone base proportion as well as covering the implants. The PLA coating was more efficient than the PLGA one and the release profile obtained with lot 2 coated with PLA-5.9 and PLA-230 was close to the optimum for 1 week release. However, after the fourth day, the release rate fell and the total amount of methadone was not completely released, probably due to the slow diffusion release of methadone clorhydrate through the hydrophobic polymer film. Therefore all the new implants were only made with methadone base.

3.2.3. Methadone base implants

The in vitro release profile of lot 4 implants (Table 2) showed that 27% was delivered in the first 24 h approximately, double the ideal for 1-week formu-

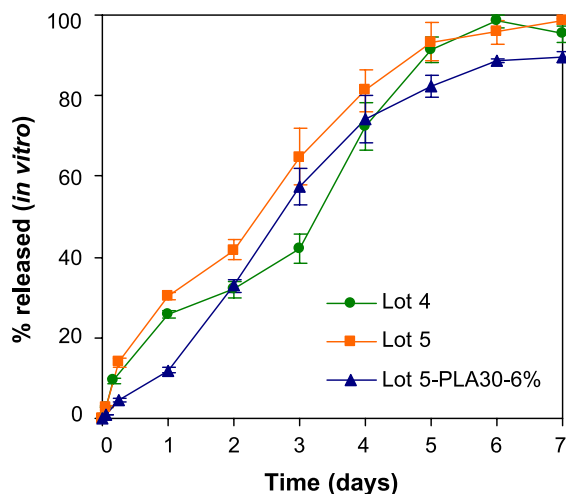


Fig. 2. In vitro release profile from different methadone base implant lots designed for 1 week release.

lation and the total load was released in about 6 days (Fig. 2).

These implants were considered adequate to be tested in vivo. The serum level–time curve obtained after the insertion of one implant from lot 4 to each mouse showed an average concentration around 400 ng/ml during the first few hours, then decreased until maintaining a level of around 100 ng/ml during 4 days, finally falling to less than 50 ng/ml afterward (Fig. 3). To establish the in vivo–in vitro correlation, these implants were extracted at the same serum point in time and the remaining methadone was determined. Fig. 4 shows an excellent correlation, with a slope nearly equal to 1.

In order to achieve and maintain higher serum levels of around 200 ng/ml, a larger methadone dose was required. A new implant lot, with the same composition but weighing 26 mg, was prepared according to the in vivo–in vitro correlation obtained with lot 4. In this occasion the blend was compressed with the 6-mm punch and a tablet was obtained, named

Table 3

Coating thickness (mm) obtained from the different polymers used at the selected concentrations in methylene chloride

Coating polymer concentration (%)	PLGA-12	PLA-5.9	PLA-30	PLA-230
6	–	0.035 ± 0.084	0.043 ± 0.011	0.067 ± 0.052
12	0.061 ± 0.034	0.056 ± 0.043	0.067 ± 0.054	0.086 ± 0.060
25	–	–	0.128 ± 0.010	–

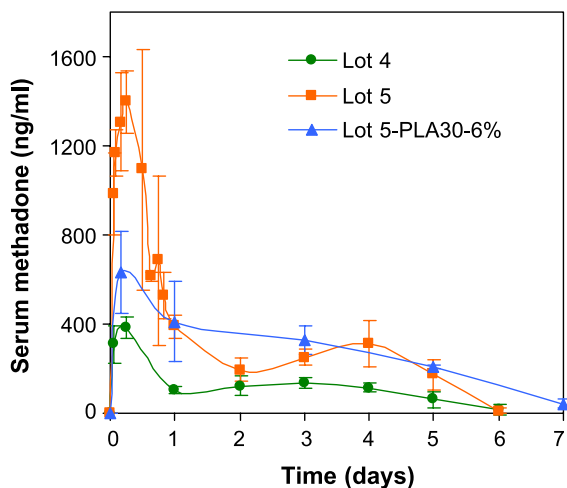


Fig. 3. Methadone serum levels obtained with different 1-week methadone base implants.

lot 5 (Table 1). The *in vitro* release profile was similar to lot 4 with a 30% release in the first day (Fig. 2) which was reflected in the serum level–time curve (Fig. 3) with high methadone concentration at the beginning and then a therapeutic range between 170 and 380 was maintained for around 6 days. The *in vivo*–*in vitro* correlation was also very good (slope close to 1) (Fig. 4). Therefore the reduction of the burst effect could lead to an optimum formulation. The implants were coated with PLA of different molecular weights (5900, 30,000 and 23,000) at two polymer concentrations (6% and 12% in methylene chloride) (Table 3) based on results from the methadone clorhydrate and methadone clorhydrate/base blends implants. The effect of the coating on the *in vitro* release profile was an important reduction in the delivery rate during the first 2 days (Fig. 2). Significant differences were not observed with the different PLA coatings ($f_2 > 50$), therefore lot 5 coated with PLA-30 at 6% in methylene chloride (lot 5-PLA30-6%) was selected for implantation in mice. The *in vivo* methadone serum levels (Fig. 3) reflected the reduction of the burst effect decreasing the initial concentration from 1300 ng/ml with lot 5 to 600 ng/ml with lot 5-PLA30-6% and maintaining serum levels between 100 and 400 ng/ml during 6 days. The *in vivo*–*in vitro* correlation obtained with this formulation is also shown in Fig. 4, the *in vitro* release perfectly reflected the expected *in vivo* release.

It has been previously suggested that the kinetics of methadone undergo adaptative changes during repeated administration of the drug due to progressive changes in the disposition process in the body [8]. Our results have confirmed time dependence in methadone kinetics. Since a drug absorption–disposition characteristic function must be linear and time invariant in order to apply deconvolution methods, we used the characteristic functions corresponding to single- and multiple-dose protocols to establish the expected *in vivo* release range. Fig. 5 shows the two extreme curves estimated by deconvolution corresponding to the three implants tested *in vivo* together with their *in vivo* release profile obtained from the remaining methadone in the implants. These release curves were always closer to the curve which was obtained by deconvolution using the single-dose characteristic function (lowest $Cl_s = 7.4$ l/h/kg) during the first 3 days, suggesting non-auto-induction with this continuous administration. It should be noted, however, that a slight tendency towards the higher curve (obtained from the multiple-dose characteristic function) can be observed.

Lot 5-PLA30-6% could be considered a good formulation for 1-week treatment. However, a longer sustained drug release system could be potentially more efficient due to the length of the treatment.

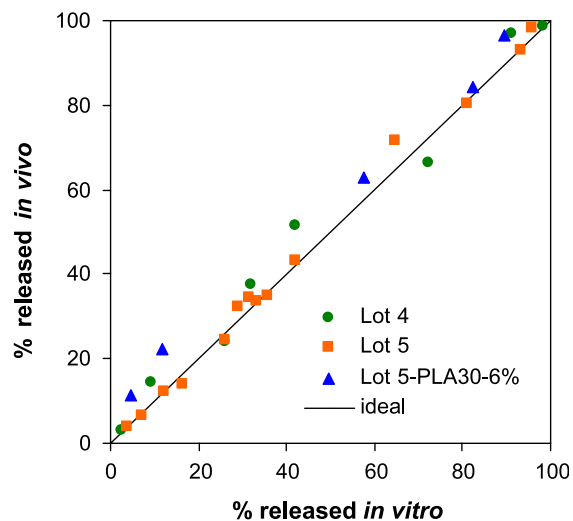


Fig. 4. *In vivo*–*in vitro* correlation obtained for different 1-week methadone base implants. The *in vivo* release percentage represented here was calculated from the remaining methadone in the implant.

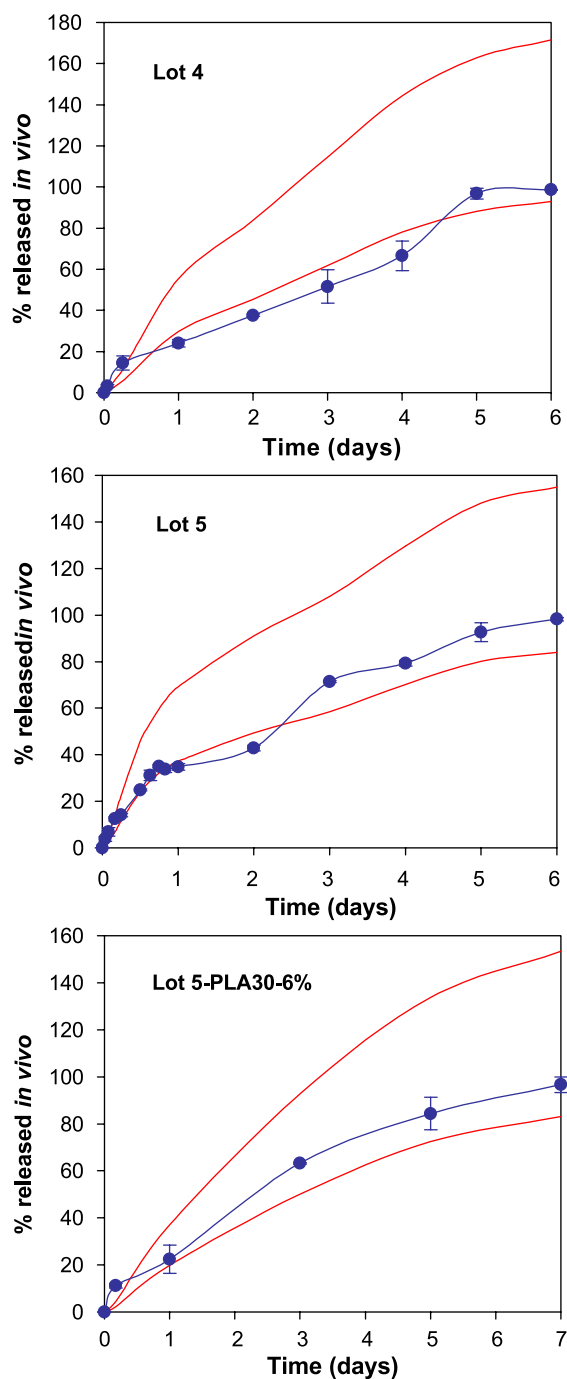


Fig. 5. In vivo release profiles of the three 1-week implants calculated from the remaining methadone together with the predicted release range obtained by deconvolution using the characteristic function corresponding to single-dose (lower curve) and multiple-dose (upper curve) protocols.

3.3. One-month implants

In this case the implants were made with 65% methadone base and 35% of PLA-30. The PLGA used in the composition of 1-week implants was changed to PLA-30 because of its higher lipophilicity. One preliminary implant lot, lot 6 (Table 2), was prepared containing 31 mg of methadone. This quantity corresponds to the methadone dose required according to the extreme clearances when levels between 100–200 ng/ml are necessary. These implants were coated with PLA-30 at 12% (lot 6-PLA30-12%) and 25% (lot 6-PLA30-25%) in methylene chloride (Table 3). The coating reduced the initial in vitro release (Fig. 6). The reduction of the burst effect was more apparent with the higher concentration coating. The implants of lot 6-PLA30-25% delivered 30% in 1 week and then the release rate fell to 17%/week during 4 more weeks, reaching a total release of about 98%. Therefore these implants maintain the release during 5 weeks although the release rate is slower than the ideal after the first week. After considering these results together with those obtained in vivo with 1-week implants, the amount of methadone was increased to 45 mg which increases the weight of the implant to 70 mg. This new variation was called lot 7-PLA30-25% (Table 2). The in vitro profile obtained showed a 20% release in the first week and around 95% in approximately 5 weeks (Fig. 6). A preliminary test was performed with a few mice

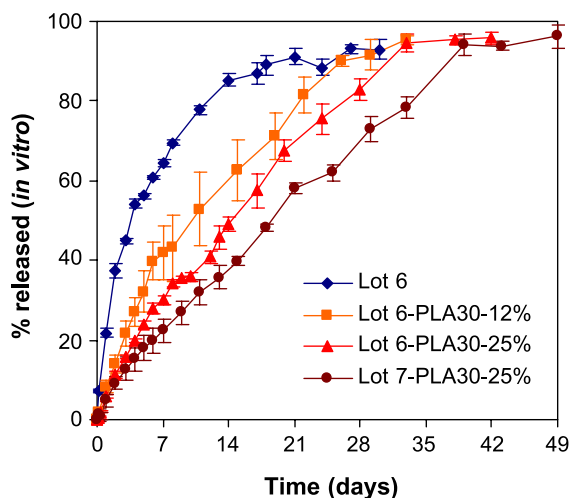


Fig. 6. In vitro release profiles from different methadone base implant lots designed for 1-month release.

before carrying out the complete *in vivo* experiments. Some serum samples were determined during 2 weeks and methadone levels were always around 100–150 ng/ml which correspond to the concentrations expected with the highest clearance. These results indicated that auto-induction could be taking place due to a longer administration. Therefore the complete *in vivo* experiment was carried out with two implants since the desired concentration should be higher, around 200 ng/ml. At the beginning serum levels (Fig. 7) of approximately 700 ng/ml were reached but, after the first 4 h, they fell and stayed between 100 and 300 ng/ml throughout 5 weeks. The *in vivo*–*in vitro* correlation was also good, with a slope of 0.952 (Fig. 8). The release curve obtained from the remaining methadone in the implants (Fig. 9) tended to the one estimated by deconvolution using the single-dose characteristic function during the first 3 days, showing the same behavior of the 1-week implants. Afterwards, there was a clear tendency to the levels predicted using the multiple-dose characteristic function (Fig. 9), confirming that the auto-induction process took place with this prolonged release system.

In summary, two systems were obtained for methadone controlled release during approximately 1 and 5 weeks, respectively. Both of them kept methadone serum levels in mice in the therapeutic range. However, it must be pointed out that pharmacokinetics of methadone in mice is quite different to that in humans,

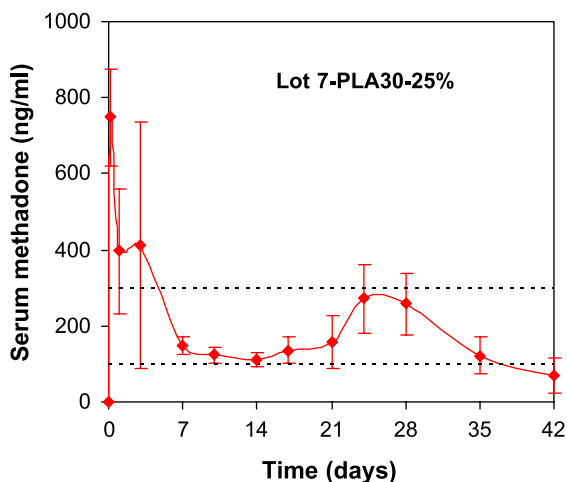


Fig. 7. Methadone serum levels obtained with two 1-month release implants.

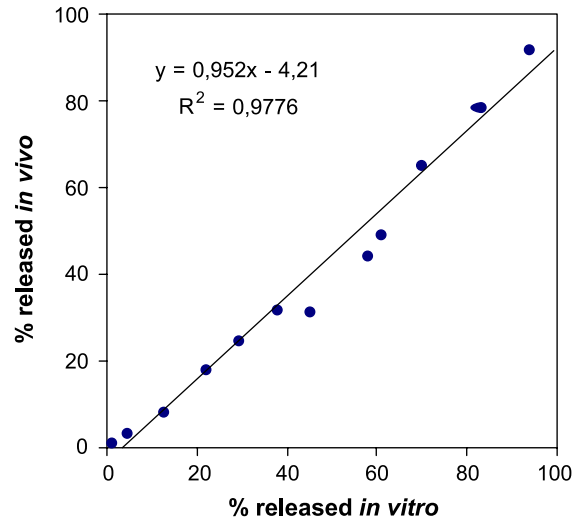


Fig. 8. *In vivo*–*in vitro* correlation obtained with the 1-month implant. The *in vivo* release percentage represented here was calculated from the remaining methadone in the implant.

showing a higher clearance and a much shorter half-life (1 h) than in humans (36 h). According to the methadone clearance for opioid addicts, the implants' weight could range between 0.4 g for 1-week treatment to 2.5 g for a month if L-methadone is used. Although these implants are too big for clinical use, a

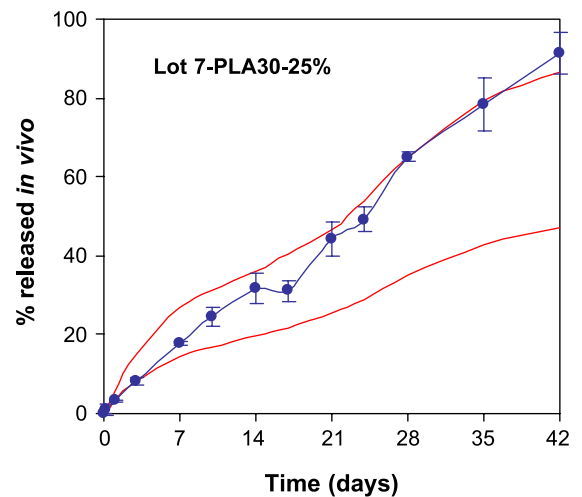


Fig. 9. *In vivo* release profile of the 1-month implant calculated from the remaining methadone together with the predicted release range obtained by deconvolution using the characteristic function corresponding to single-dose (lower curve) and multiple-dose (upper curve) protocols.

constant release rate has been obtained coating the implants. Therefore the optimization of the coating thickness could allow the polymer percentage to be reduced in the formulations and smaller implants could be prepared for human use.

4. Conclusions

Two implants have been obtained which are able to release methadone during 1 and 5 weeks with nearly zero-order rates. A good *in vivo*–*in vitro* correlation was found, with a slope approximately equal to 1, allowing the *in vivo* released profile to be predicted from the *in vitro* assays. In addition, since an increase of methadone clearance after continuous administration has been shown, the deconvolution methods to predict the *in vivo* release profile cannot be used. However, the *in vivo* release profile range has been estimated using the single- and multiple-dose absorption–disposition function.

In conclusion the implants developed could potentially be efficient for methadone maintenance treatment since therapeutic concentrations are achieved and maintained during a long period of time although the implant size could be a limiting factor.

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