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# Optimization of 7-day release (in vitro) from DL-PLA methadone microspheres

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**Abstract**

Poly(DL-lactic)acid-methadone base microspheres able to release the drug over 7 days have been developed using the solvent evaporation method and an optimization strategy. The first optimization step involved in vitro release study of nine different formulations elaborated in agreement with a composite rotable second order experimental design. Polymer degradation was observed during microsphere preparation; degradation index, defined as the number of broken bonds in relation to the initial number of polymer molecules, was less than 0.72 for weight average molecular weight (Mw) below 145 000 and 0.93 for 198 000 Mw. Degradation index ranging between 1.4 and 6.54 were observed during in vitro release assay. Specific surface determinations show larger than expected values from the number-volume average diameters, and DSC studies showed that methadone is dissolved to a limited extent. Once the region of interest was located, two new formulations were prepared and tested. Optimum formulation released 18.6% of drug content over the first 24 h, against a targeted value of 14.3% for a near zero order release rate over 7 days, and 68.0% after 7 days.

*Keywords:* Microspheres; Methadone; DL-PLA; Optimization; Controlled release; Degradation index

## 1. Introduction

Methadone is a synthetic analog of morphine used for pain relief and opiods addiction treatment, and it is currently available as L, active, and in DL forms. Due to the chronic treatments with methadone, it has been the object of studies oriented to the development of sustained release forms as microspheres. Cha and Pitt (1988) developed L-methadone biodegradable microspheres able to release about 80% of the drug in vitro in 7 days following a near zero order release kinetic. However, the complex mixture of microsphere types used by these authors, poly($\epsilon$-caprolactone-co-L-lactic)acid, (PCL-LA) with 85% of L-lactic acid (LA), plus PCL-LA with 95% of LA and plus poly(L-lactic acid) (PLLA) in proportion 11:48:41, as well as the large size of the microspheres, between 50 and 200 $\mu$m for PCL-LA microspheres, and 212-500 $\mu$m for PLLA microspheres, made them unsuitable for parenteral administration due to their large size. The development of

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more easily made biodegradable microspheres was therefore suggested.

In this paper we approach the same objective, the development of biodegradable microspheres for 1-week methadone delivery, but using a different strategy: only DL-PLA was used in order to simplify the manufacture process, and the fraction of polymer in microspheres was limited to a maximum of 85% to keep the amount of microspheres to be administered within reasonable limits; moreover, the diameter of microspheres must be small enough to ease the parenteral administration and favour polymer degradation, once the drug has been released.

## 2. Materials and methods

### 2.1. Experimental design and statistical analysis

The control variables used to optimize the release rate were polymer content expressed as % ($X_1$) and polymer weight average molecular weight, Mw ($X_2$). The optimization method was the surface response one (Box and Draper, 1986). This method is based on a second order polynomial function fitted to the data points obtained using a second order rotable experimental design (Cochran and Cox, 1980). For this purpose, nine different formulations of DL-methadone were prepared (coded as A to I; see Table 1 and Table 2). Five replicates of the experimental central point (I-1 to I-5) were also prepared in order to have a measure of the error involved in the elaboration and testing of microspheres.

Table 1
Levels and actual values of control variables and polydispersivity of DL-PLA

| Level | Polymer | | |
|---|---|---|---|
| | Content ($X_1$) | Mw ($X_2$) | Pd |
| −1.414 | 50.0 | 33 200 | 1.57 |
| −1 | 54.4 | 63 000 | 1.26 |
| 0 | 65.0 | 100 000 | 1.49 |
| 1 | 76.6 | 143 800 | 1.50 |
| 1.414 | 80.0 | 195 800 | 1.61 |

### 2.2. Microspheres preparation and characterization

Five batches of DL-PLA of different molecular weights were prepared using the ring opening method (Kulkarni et al., 1966). Time, temperature and catalyst concentration were chosen in agreement with previous data available (Munguía et al., 1992). Microspheres were prepared using the solvent evaporation technique: variable amount of DL-PLA (2.00–3.78 g) and *dl*-methadone (0.80–2.28 g) were dissolved in dichloromethane and poured over 1 l of 0.1% polyvinyl alcohol in water. Emulsification was done using a turbine homogenizer (IKA mod. Ultra-Turrax T-25, head type KR) at 8000 rev./min during 5 min. Solvent evaporation was completed in the following 5 h at room temperature and atmospheric pressure using a paddle stirrer (Turú Grau, model D-6) at 250 rev./min. Microspheres were collected by centrifugation and freeze-dried.

DL-PLA molecular weight was determined by gel permeation chromatography (GPC) (Waters system with one Model 510 pump, Rheodyne injector, Model 410 differential refraction index detector, oven for columns and Maxima 820, chromatography software v.3.30 for data adquisition). The conditions of GPC analysis were: four columns (Ultrastyragel) of 500, 1000, 10 000, and 100 000 Å of pore size, oven temperature equal to 31°C, and tetrahydrofuran (Merck) with flow rate equal to 0.9 ml/min. The system was calibrated using monodisperse polystyrene (Tokyo Soda Ltd.) with molecular weights 2800, 5570, 10 300, 43 900, 102 000, 190 000, 355 000 and 710 000. Sample concentration and volume were 0.5% and 20 $\mu$l, respectively.

Methadone content (dissolution with $Cl_2CH_2$, and UV determination at 290 nm), particle size analysis (Coulter mod. Multisizer II), specific surface area (Micromeritis Mod. Asap 2000), absolute density (Quantachrome Mod. MPY-2), differential scanning calorimetric (Netzsch, mod. STA 409 EP), and release rate were used in order to characterize the microspheres. Release rate studies were conducted from 40 mg of microspheres in 0.066 M phosphate buffer pH 7.4 and $\mu$ = 0.264 with 0.001% of polysorbate 80. Each

Table 2
Summary of experimental results: methadone trapping relative to theoretical content (trapping effic.), weight average molecular weight (Mw) and polydispersivity (Pd) of polymers before and after release test

| Lot | Variables | | Trapping effic.(%) | Polymer-before | | Polymer-after | |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | | Mw $10^{-3}$ | Pd | Mw $10^{-3}$ | Pd |
| A | −1 | −1 | 84.1 | 58.9 | 1.28 | 21.5 | 1.93 |
| B | 1 | −1 | 70.3 | 58.9 | 1.35 | 16.6 | 1.85 |
| C | −1 | 1 | 78.3 | 101.7 | 1.58 | 16.5 | 1.99 |
| D | 1 | 1 | 71.7 | 108.9 | 1.61 | 16.6 | 1.87 |
| E | −1.414 | 0 | 94.8 | 71.3 | 1.83 | 18.0 | 2.14 |
| F | 1.414 | 0 | 91.7 | 71.6 | 1.53 | 32.5 | 2.54 |
| G | 0 | −1.414 | 82.2 | 25.4 | 1.47 | 12.1 | 1.70 |
| H | 0 | 1.414 | 99.0 | 100.3 | 1.60 | 21.8 | 2.62 |
| I1 | 0 | 0 | 91.4 | 78.0 | 1.47 | 25.6 | 2.50 |
| I2 | 0 | 0 | 85.3 | 87.3 | 1.45 | 32.2 | 2.45 |
| I3 | 0 | 0 | 94.9 | 77.7 | 1.57 | 35.9 | 2.77 |
| I4 | 0 | 0 | 100 | 80.7 | 1.50 | 37.6 | 2.23 |
| I5 | 0 | 0 | 86.6 | 79.4 | 1.54 | 39.0 | 2.11 |
| J | 1.471 | 1 | 75.8 | 123.0 | 1.66 | 11.7 | 1.56 |
| K | 1 | 1.414 | 80.5 | 86.4 | 1.60 | 14.5 | 1.75 |

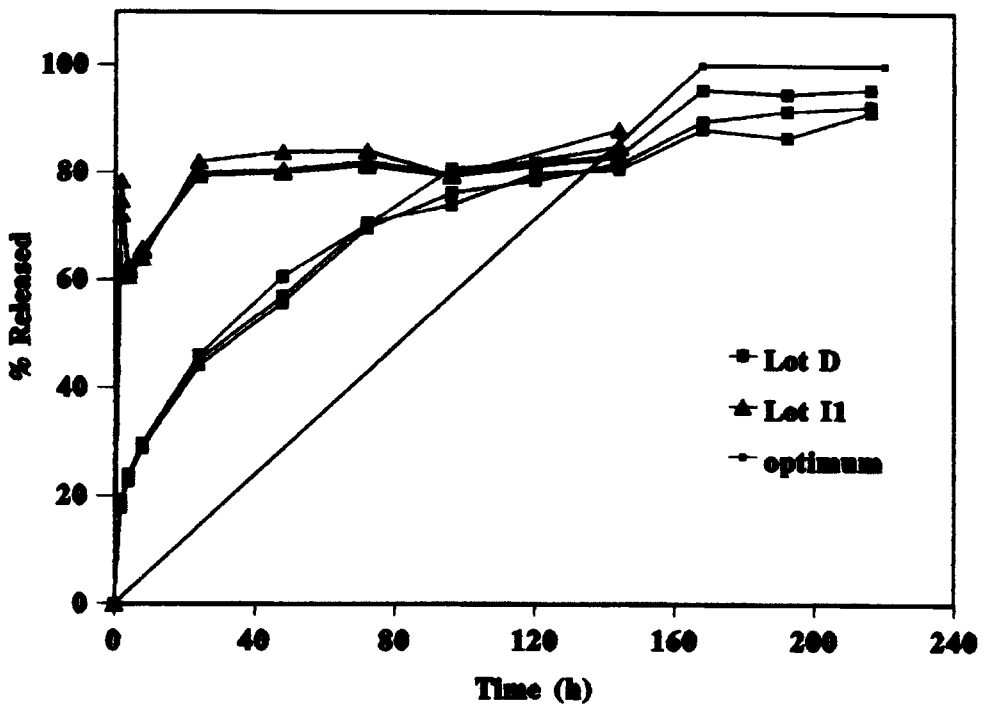


Fig. 1. Percentage of dose release vs. time for formulations D and I1. Solid line corresponds to the target release rate, equal to 14.3%/day.

assay was replicated three times. The remaining conditions were: conical flask of 100 ml nominal capacity, 37°C and stir rate equal to 50 rev./min (magnetic bar length 2.3 cm).

## 3. Results and discussion

As was stated previously, the release rate optimization goal was to reach a constant methadone release over a period of 7 days, i.e., about 14% of the dose per day. Because of the lack of an appropiate kinetic model to explain the release rate data from all the formulations involved in this study, three direct measurements from release curves were used to quantify this process: percentage of dose released over first 24 h ($D_{24}$), percentage of dose released over 7 days ($D_{168}$), which must be near 100%, and mean release time (MRT). The first one was chosen because methadone half-life in humans permits once-a-day oral admistration to control abstinence symptoms, and therefore the burst effect measured over a shorter period of time is not relevant from the therapeutic point of view. Mean release time is a priori unrelated with both $D_{24}$ and $D_{168}$, and a target value equal to 84 h is expected for a zero order release rate over 7 days.

Table 2 shows basic experimental results together with coded values of control variables. Fig. 1 shows the best and one of the worst formulations, D and I1 respectively. In spite of the $D_{24}$ reduction reached with formulation D, 45.9%, this figure is far from the target value. Table 3 sumarizes the experimental results for the parameters chosen for the release rate optimization. The fitted equations for $D_{24}$ and $D_{168}$ parameters using the quadratic models were:

$$D_{24} = 81.96 - \underset{(3.107)}{11.75X_1} - \underset{(3.107)}{0.453X_2} - \underset{3.331)}{5.428X_1^2} - \underset{(3.331)}{10.058X_2^2} + \underset{(4.392)}{0.908X_1X_2}$$

$$D_{168} = 90.88 - \underset{(1.656)}{5.119X_1} + \underset{(1.656)}{1.031X_2} - \underset{(1.776)}{0.999X_1^2} - \underset{(1.776)}{2.284X_2^2} + \underset{(2.342)}{4.373X_1X_2}$$

where $X_1$ and $X_2$ are the coded variables (standard deviation of estimates between brackets). The correlation coefficient $r^2$ was 0.78 for $D_{24}$ and 0.68 for $D_{168}$; this means that 78% and 68%, in each case, of the overall variability is explained by the model, and the lack of fit test was not significant. Although only linear coefficients for the first variable, $X_1$, and in the case of $D_{24}$, the quadratic term with respect to the second variable, $X_2$, are statistically significant, we have used the overall equation since zero is not an unbiased estimator of the non-significant terms.

The surface responses for $D_{24}$ and $D_{168}$ are depicted in Fig. 2. The fitted model for $D_{24}$ variable shows a maximum in-point ($-1.1$, $-0.1$) instead of a minimum as would be desirable, and therefore, in agreement with quadratic model properties, the minimum value will be located at the boundaries; lowest $D_{24}$ values can be reached with higher polymer content, both for high and low molecular weight. $D_{168}$ surface response has a saddle-point outside the experimental range of both variables, but values over 90% are predicted for both high polymer content and high molecular weight. MRT analysis was excluded because no relationship with control variables was found. These data suggest that formulations with a re-

Table 3
Percentage of methadone released over the first 24 h ($D_{24}$), mean release time (MRT), and percentage of methadone release over 7 days ($D_{168}$) from the different lots of microspheres included in the experimental design and lots J and K (Mean values of three test)

| Lot | $D_{24}$ (%) | MRT (h) | $D_{168}$ (%) |
|---|---|---|---|
| A | 81.0 | 7.6 | 93.4 |
| B | 48.5 | 26.0 | 77.1 |
| C | 73.8 | 47.6 | 89.9 |
| D | 45.0 | 50.7 | 91.1 |
| E | 87.1 | 7.4 | 97.8 |
| F | 63.9 | 13.3 | 79.5 |
| G | 63.7 | 14.0 | 86.8 |
| H | 68.8 | 22.2 | 85.3 |
| I1 | 79.9 | 9.8 | 85.0 |
| I2 | 77.6 | 6.8 | 88.1 |
| I3 | 73.1 | 15.3 | 91.0 |
| I4 | 93.2 | 8.1 | 99.6 |
| I5 | 86.0 | 12.5 | 90.7 |
| J | 10.4 | 185.6 | 85.4 |
| K | 18.6 | 92.2 | 75.6 |

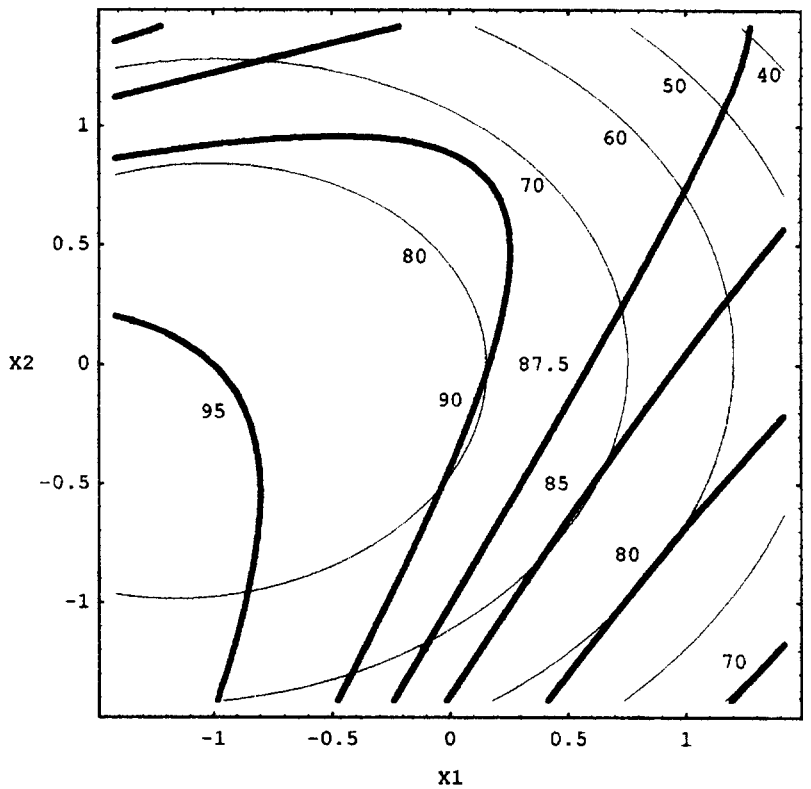


Fig. 2. Surface responses of percentage of dose released over first 24 h, $D_{24}$ (———), and percentage of dose released over 7 days, $D_{168}$ (——), vs. percentage of polymer in microspheres (X1) and Mw (X2), both as coded variables. Coefficients of variation were 9.5% and 6.0% respectively.

duced $D_{24}$ values and above 90% for $D_{168}$ can be obtained using microspheres with high polymer content and high molecular weight.

Physical interpretation of these results presents some difficulties because different processes are involved in methadone release. As can be seen from Tables 1 and 2, there is a reduction in polymer Mw during microsphere preparation. It is well-known that the hydrolysis of polyesters is subject to general and specific acid catalysis, but basic catalysis is poorly understood (Cha and Pitt, 1988, 1989), and methadone, as well as other basic drugs, can catalyze PLA degradation. PLA chain cleavage can also be induced by ultrasounds (Kost et al., 1989; Cro et al., 1992). Also, a synergic action of the media catalytic effect with high shear stress have been studied for polymers other than PLA (Rudakova and Zaikov, 1988). Therefore, a combined effect of catalysis and high shear chain cleavage cannot be excluded. To study the extent of polymer degradation it is convenient to use the degradation index, *DI*, instead of number or weight average molecular weight, because the first parameter gives us information about the extent of chain scission. *DI* is defined as the number of broken bonds relative to the initial number of polymer molecules (Glynn et al., 1976). *DI* after time $t$ has elapsed can be calculated using the formula

$$DI = \frac{M_n^0}{M_n^t} - 1$$

where $M_n^0$ and $M_n^t$ are the number average molecular weights at time zero and after time $t$ respectively. Fig. 3 shows the *DI* during microsphere manufacture of formulations A to I5. As can be seen the *DI* increases slightly with the polymer Mw, but there is a clear polymer degra-

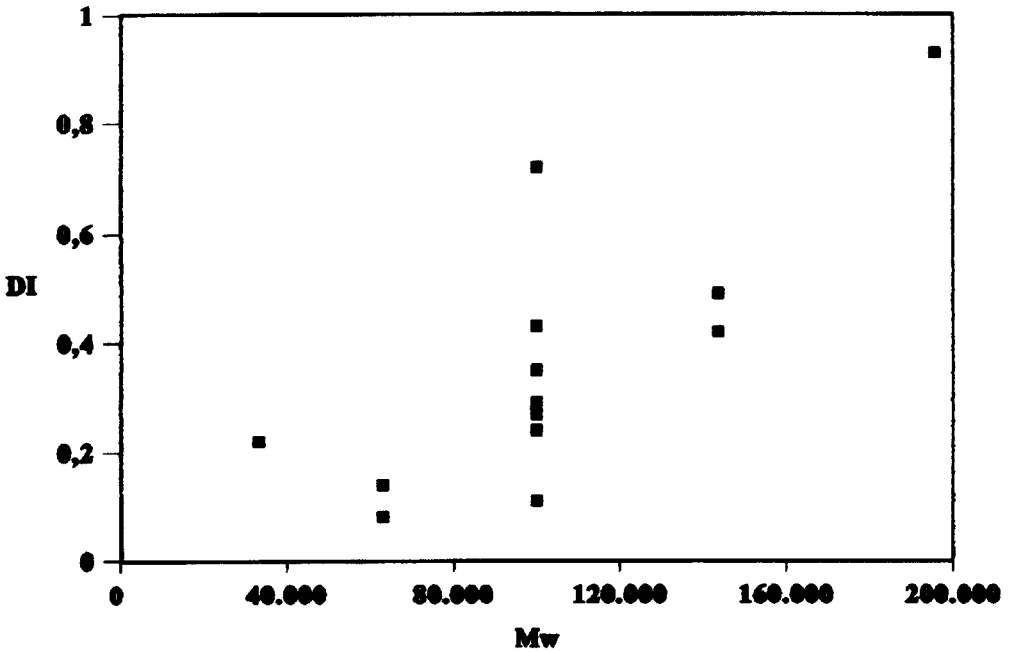


Fig. 3. Degradation index (*DI*) during microspheres elaboration.

Fig. 4. Microphotograph of DL-PLA methadone base microspheres, lot F.

dation for the higher Mw polymer due to the selective scission of high molecular weight chains since they are under higher tension than shorter ones (Delgado, 1995). Therefore for all lots manufactured, actual values of polymer Mw in microsphere are not the same as those of polymers used (Tables 1 and 2). However, it is not affected by the methadone content, at least in the range of concentrations used, probably because they are higher than the necesary amount to show its catalytic effect. On the other hand, all the microsphere lots are similar morphologically, presenting

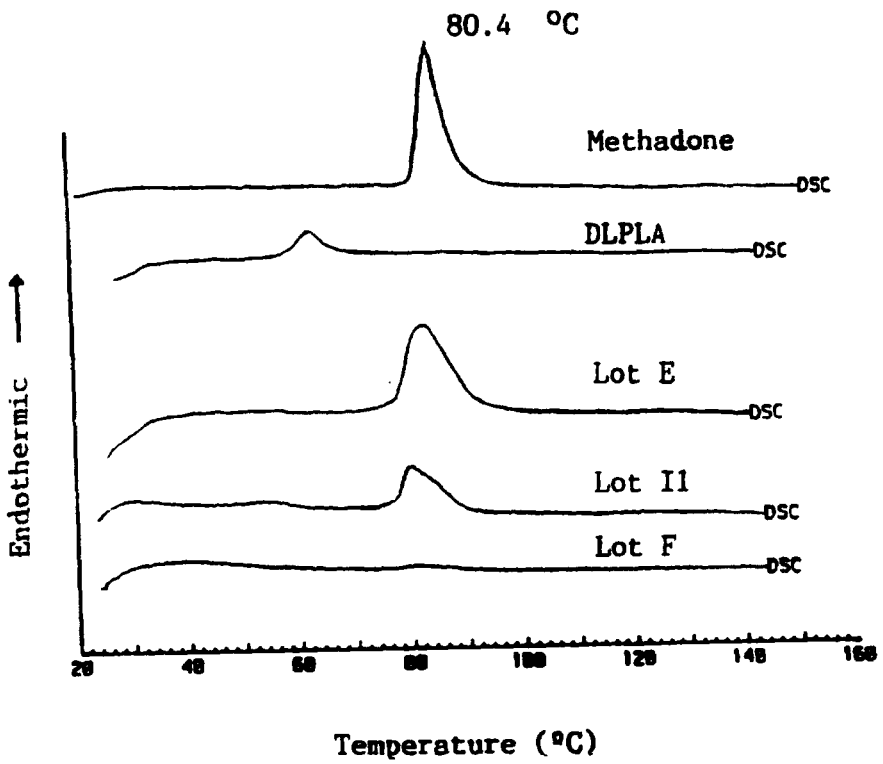


Fig. 5. The DSC thermograms of methadone base, DL-PLA Mw 100 000 and three lots of microspheres elaborated with this polymer: lot E (47.4% methadone), lot I1 (32% methadone), and lot F (18.3% methadone).

spherical shapes and apparently smooth surfaces as can be seen in Fig. 4.

DSC from methadone base, DL-PLA and three formulations, E (with an actual proportion of methadone equal to 47.4% w/w), F (18.3%) and I1 (32%), are depicted in Fig. 5. Both methadone base melting point and DL-PLA vitreous transition temperature are clearly identifiables. Comparing DSC from the three formulations we can conclude that methadone solubility in PLA must be around 18.3% w/w. Excess of methadone above this figure is not dissolved in the polymer, and thus is readly available to release; note that both $D_{24}$ and $D_{168}$ are ranked in the same order as in DSC peak area of methadone in DL-PLA: E > I1 > F.

Another factor that can contribute to increase the burst effect is the larger than expected superficial area. DL-PLA absolute density determinations yields values between 1.20 and 1.27 g/ml for polymer Mw of 27 600 and 195 800; 1.10 g/ml for methadone, and 1.23 g/ml for a four-lot pool of central point formulations. Particle size analysis using an electrozone counter showed a normal-log distribution and small variability (Table 4) with number-volume average diameters between 9 and 16 $\mu$m for the lots of the central point (I1 to I5), which corresponds roughly to a surface area between 0.58 and 0.37 $m^2/g$. However, the $N_2$-adsorption method gives 2.48 $\pm$ 0.143 $m^2/g$. This large discrepancy has been observed previously by Kishida et al. (1990), and it could be another determinant for the fast initial drug release.

DL-PLA degradation also takes place during in vitro methadone release (Table 2). During the release process the *DI* is higher than in elaboration because the first one takes longer, while during elaboration water difussion into the microspheres is less due to the presence of the

organic solvent, but the microspheres still keep their structure at the end of the release assay (Delgado, 1995), and our results suggest that most of the methadone release during the first 7 days is not related to polymer degradation.

Keeping in mind these results, two new microspheres formulations coded as J and K were prepared and tested (see botton of Table 2). In both cases the methadone is dissolved in the polymer matrix and the microspheres show a similar mean volume diameter: 10.25 $\mu$m for lot J and 11.81 for lot K. Fig. 6 shows average release curves from these formulations together with the best formulation from the first step (formulation D) and target cumulative release rate. Good agreement between experimental and target release was found for 0–5 days interval for formulation K, but these new formulations (J and K) release only 42% and 68%, respectively, of the methadone content during the 7 days, while formulation D release the 90%. Cha and Pitt (1989) found similar results, suggesting that the immobilization of methadone by the polymer carboxilic groups is responsible for the incomplete release.

Table 4
Number-volume average diameters corresponding to accumulated percentages of 16%, 50% and 84% for the different microsphere lots included in the experimental design

| Percentil < | 16 | 50 | 84 | $\sigma_g$ |
|---|---|---|---|---|
| Lot | particle size ($\mu$m) | | | |
| A | 8.48 | 11.14 | 14.19 | 1.29 |
| B | 10.48 | 12.63 | 14.63 | 1.81 |
| C | 10.96 | 14.62 | 18.33 | 1.29 |
| D | 8.81 | 12.18 | 16.50 | 1.36 |
| E | 9.36 | 14.26 | 20.98 | 1.49 |
| F | 9.31 | 13.84 | 25.88 | 1.66 |
| G | 4.69 | 16.04 | 35.41 | 2.74 |
| H | 11.57 | 18.78 | 27.04 | 1.52 |
| I1 | 6.45 | 8.95 | 12.01 | 1.36 |
| I2 | 6.88 | 10.09 | 14.59 | 1.45 |
| I3 | 9.29 | 15.62 | 25.28 | 1.65 |
| I4 | 5.64 | 9.61 | 19.36 | 1.85 |
| I5 | 7.65 | 12.78 | 19.65 | 1.60 |

Anyway, the release rate from formulations J and K is slower than the model predicts, probably because the areas in the which we are working in both response surfaces ($D_{24}$ and $D_{168}$) are the boundaries where the model prediction error is larger. Although the delay in release rate presented by formulation J cannot easily be justified for this reason only and possibly, in this case, the methadone charge is so diminished that diffusion though the polymeric matrix requires a larger run, the rate of water access into the matrix is also decreasing.

## 4. Conclusions

These results show that the manufacture of *dl*-methadone microspheres for weekly administration is feasible keeping both microsphere size and drug content inside an acceptable range; DL-PLA Mw 195 800 and 80% of polymer permitted burst effect control, but incomplete drug release arose. Furthermore the results suggest that the release of methadone in excess over their solubility in the polymer, is controlled both by dissolution/diffusion, while dissolved drug release is controlled by molecular diffusion through polymer matrix and polymer degradation. The importance of this second mechanism depends on the proportion of undissolved methadone, because once this fraction has been released, easier access to dissolved fraction is possible. Therefore, in vivo bioavailability of formulations J and K will depend on the rate of chain scission, and therefore an in vivo evaluation is compulsory.

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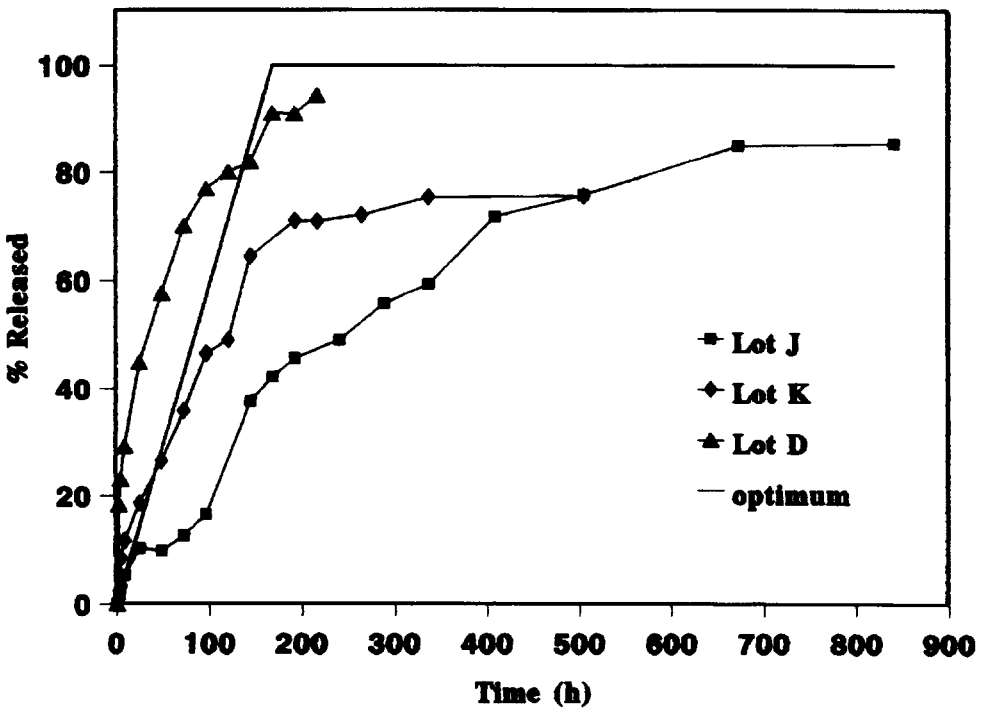


Fig. 6. Percentage of dose release vs. time for the optimized formulations (J and K) and formulation D. Solid line corresponds to the *target* release rate, equal to 14.3%/day. Average within time coefficients of variation range from 3% to 11%.

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