Research Article

Meloxicam Depot Parenteral Bio-Degradable Microspheres: Preparation, Characterization and *In-Vivo* Evaluation

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ABSTRACT

The demand for a sustained-release MX delivery system for the treatment of rheumatoid arthritis is clear. This was the driving force for the study. The aim of this work was to utilize the biocompatibility characteristics of the biodegradable polymers viz. poly lactide-co-glycolide (PLGA) and poly ε-caprolactone (PCL) to prepare sustained release injectable microspheres of the anti-inflammatory drug Meloxicam (MX). MX microspheres were prepared using O/W emulsion-solvent evaporation/extraction method. The influence of the formulation parameters on the characteristics of the prepared microspheres was investigated to achieve formulation with suitable injectable size, high encapsulation efficiency, and sustained drug release with minimal burst release. Selected microspheres formulation was injected in an animal model to determine MX plasma level and compared with MX suspension. Results showed that microspheres prepared using PCL-PLGA blend showed improved sustained release pattern with lower initial burst. Formula F41 was selected the optimum formula, with 20% w/v PCL in PLGA and drug: polymer ration 1:2.5 and showed 72.3% of yield and encapsulation efficiency % (EE%) of 93.3 %. *In Vivo* studies showed that selected microspheres formula from polymer blend (formula F 41) when injected into rats showed therapeutic MX levels achieved for 21 successive days.

Keywords: Meloxicam, Biodegradable polymers, microencapsulation, depot parentral preparation.

INTRODUCTION

Meloxicam (MX), Fig. 1, is an anti-inflammatory drug of the oxicam group derivative[1]. MX inhibits is relatively a selective inhibitor of cyclo-oxygenase-2 (COX-2), which has analgesic, antipyretic and anti-inflammatory properties[2]. It is preferentially selective to COX-2 inhibition, therefore at high doses; it may bind to COX-1 resulting in a decrease in the production of physiologic prostaglandins [3].

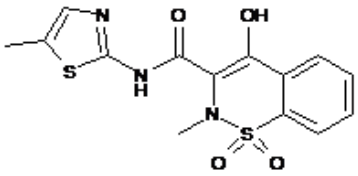

Figure 1: Chemical structure of MX

Biodegradable Alpha-hydroxy esters polymer class, particularly the copolymer poly lactide-co-glycolide (PLGA) and polycaprolactone (PCL), have shown great interest in the field of controlled drug delivery [4-6]. Sustained-release parenteral drug delivery systems provide advantages when compared to conventional counterparts. These advantages include (a) increased bioavailability, (b) Accurately control drug release rates over prolonged periods of time, following single administration, (c) in addition to localized drug delivery, minimizing undesired effects of the drug. The fabrication of biodegradable systems in the form of microspheres enhances powder flowability and minimizes the pain associated with device implantation. The use of biodegradable polymers in such systems provides an added asset, due to their enhanced tissue biocompatibility[7], and the lack of necessity for surgical removal thus improving patient compliance[8].

This work aimed at preparation of sustained release parenteral dosage form by encapsulating the non steroidal anti-inflammatory drug MX into biodegradable polymers, PLGA and PCL, as microspheres. In addition, study the influence of the formulation parameters on the characteristics of the prepared microspheres. This is an essential step to obtain microspheres formulation, with suitable injectable size, improved encapsulation efficiency, and sustained drug release over a long period (depot effect), with minimal burst release. This work also aimed at applying the prepared microspheres formulation in an animal model, to determine the MX plasma level.

MATERIALS AND METHODS

Materials

PLGA Resomer RG 502 H, MW 17000 Da, was purchased from Boehringer Ingelheim (Germany). Poly(ε-caprolactone) (PCL) (MW 10 kd) and isopropyl myristate (IPM) were purchased from sigma Aldrich (USA). MX was kindly granted from Medical Union Pharmaceuticals (MUP) (Egypt). Polyvinyl alcohol (PVA) was purchased from Fluka Chemie GmbH, Germany. Polyethylene glycol (PEG) 6000, gelatine, were purchased from ADWIC, Egypt. All other chemicals were of analytical grade.

Methods

Preparation of MX Microspheres

Emulsion solvent-evaporation (ESE) method was used for MX Microspheres preparation. Different weights of MX

were added to the polymer-dichloromethane (DCM) solution and sonicated for 5 minutes. Additives, IPM and PEG 6000 when present, were dissolved prior to the addition of drug. The organic phase was added dropwise to 50 ml the emulsifier solution stirred at 2000 rpm for 10 minutes. The formed emulsion was then stirred for further 3 hours at 400 rpm. The produced microspheres were collected by centrifugation at 8000 rpm, washed 3 times with distilled water and then lyophilized overnight (FreeZone 18, Labconco, USA). In case of PCL and PCL-PLGA microspheres, the same procedure was used and 150 ml quench water was added immediately after stirring the formed emulsion at 400 rpm. Without the quench water, the microspheres did not form.

Characterization of MX Microspheres

Morphology

The microspheres surface morphology was evaluated by scanning electron microscopy (SEM) (Jeol JSM-5400 LV, Jeol LTD, Tokyo, Japan). Microspheres were spread on a carbon double-adhesive layer on a metal holder and gold-coated using Ion-sputtering device.

Particle Size Analysis

The size distribution of the formulated microspheres was investigated using laser light diffraction (Cilas, France). All samples were analyzed 5 times and average results were taken. The 10% (D_{10}), 50% (D_{50}) and 90% (D_{90}) sizes where the microspheres fell were used to characterize the microsphere size distribution. The mean diameter was taken as the average of D_{10}, D_{50}, and D_{90} values. Span value was used to represent size uniformity and dispersity of the microspheres [9, 10], it was calculated from the following formula:

$$Span = \left[\frac{D_{90} - D_{10}}{D_{50}}\right] \quad (1)$$

Encapsulation Efficiency

Weighed amounts of the microspheres were dispersed in 50 ml DCM and then sonicated for 10 minutes to dissolve the microspheres. Aliquots of the organic solution were analyzed in DCM at 340 nm. Drug encapsulation efficiency percent (EE %) was calculated using the following formula:

$$EE\% = \frac{\text{actual amount of drug in microspheres}}{\text{theoretical amount of drug in microspheres}} X\ 100 \quad (2)$$

Drug Release Study

Drug-loaded microspheres (10 mg) suspended in 7 ml phosphate buffered saline (PBS) were kept in a shaking water bath at 37° C. Release experiments were carried out under sink conditions. At pre-determined time intervals, the tubes were centrifuged, and 6 ml were withdrawn from each tube and replaced with 6 ml of fresh buffer. The drug concentration was determined in PBS at 364 nm.

***In-Vivo* Study**

Animals

Adult male rats weighing 200-250 gm were used. The animals were supplied from the National Research Centre, Giza, Egypt. The animal care and handling was carried out according to the local ethical Committee protocol, Minia University, Minia, Egypt. Rats were randomly classified into three groups. The first group, untreated control, received intramuscular sterile isotonic saline solution containing sodium carboxymethylcellulose and Tween 80. The second group was injected intramuscularly by the selected formula prepared under sterile conditions. The third group was injected intramuscularly by drug suspension. The dose was 30 mg of MX per/ kg. Plasma samples were analyzed using HPLC method for intact MX after extraction with diethyl ether according to the method described by Bae et al., 2007 [11].

Pharmacokinetic Analysis (Data Analysis)

MX pharmacokinetics parameters were assessed to the suitable model using WinNonlin™ standard version (1.5) software.

RESULTS

ESE method was selected among the different methods employed to prepare polymeric drug-loaded microspheres. This method is suitable for encapsulating lipophilic drugs[12]. Tables (1 & 2) show the different microspheres formulae prepared by ESE method with the use of various formulation parameters.

Morphology

Scanning electron microscope images of the PLGA and/or PCL formulae showed that almost all the microspheres were spherical in shape. Microspheres formulae F1 & F26 with low drug loading and no additives revealed smooth and almost intact surfaces (Fig. 2.). On the other hand, formula F3 with higher drug loading values showed corrugated surfaces as a result of the presence of drug crystals interspersed with the smooth polymer surface. Results revealed that, use of PVA or gelatine as emulsion stabilizer significantly decreased the mean size of the formulated microspheres (F26 & F35, Fig. 2.). However, no change in surface smoothness was observed. The smoothness of microspheres surface was lost by the addition of channelling agent, 10% IPM (F6 and F31) or 30% PEG 6000 (F16 & F40). Fig. 2. (F43) shows microspheres formulated using physical blend of PLGA and PCL, the microspheres revealed smooth and intact surface.

Table 1: Formulation Parameters and Properties of PLGA Loaded MX microsphere Formulae

F. No.	PLGA %w/v	emulsifier %w/v	D:P ratio	additives (%w/w)	Yield %	% content ± SD [a]	EE% ± SD [a,b]
1	7.5	Gelatin 1	1:4	-	69.5	17.15 ± 0.25	85.75 ± 4.96
2	7.5	Gelatin 1	1:3	-	65	23.5 ± 0.5	94 ± 2.06
3	7.5	Gelatin 1	1:2.5	-	47.61	27 ± 0.5	94.45 ± 1.96
4	7.5	Gelatin 1	1:2.5	2.5 IPM	56.76	26.5 ± 0.85	92.9 ± 5.01
5	7.5	Gelatin 1	1:2.5	5 IPM	50	26.12 ± 1.86	91.6 ± 5.22
6	7.5	Gelatin 1	1:2.5	10 IPM	40	25.93 ± 0.65	90.8 ± 5.22
7	7.5	Gelatin 2	1:2.5	10 IPM	82	24.73 ± 1.18	85.75 ± 4.96
8	7.5	Gelatin 3	1:2.5	10 IPM	83.3	23.29 ± 0.25	82.5 ± 4.11
9	7.5	MC 1	1:2.5	10 IPM	69	22.13 ± 0.94	77.8 ± 5.2
10	7.5	PVA 1	1:2.5	10 IPM	78	25.92 ± 1.02	91.2 ± 5.61
11	7.5	SDS 1	1:2.5	10 IPM	64.2	26.21 ± 1.36	92.1 ± 5.92
12	5	Gelatin 1	1:2.5	10 IPM	61.9	25.05 ± 0.6	88.05 ± 2.07
13	2.5	Gelatin 1	1:2.5	10 IPM	66	24.42 ± 1.86	85.62 ± 5.59
14	7.5	Gelatin 1	1:2.5	10 PEG	45.6	21.54 ± 0.54	75.15 ± 2.15
15	7.5	Gelatin 1	1:2.5	20 PEG	35	20.95 ± 0.45	73.4 ± 5.35
16	7.5	Gelatin 1	1:2.5	30 PEG	23.8	20.62 ± 0.5	72.3 ± 1.95
17	7.5	Gelatin 2	1:2.5	-	71.4	26.79±2.31	93.8±3.41
18	7.5	Gelatin 3	1:2.5	-	76.2	27.39±3.55	95.9±2.79
19	7.5	MC 1	1:2.5	10 PEG	73.2	26.88±1.23	94.1±1.13
20	7.5	MC 1	1:2.5	2.5 IPM	77.6	23.78±2.62	83.26±4.33
21	7.5	PVA 1	1:2.5	5 IPM	87.7	25.77±3.21	90.2±4.4
22	7.5	Gelatin 1	1:4	10 IPM	88.3	17.18±4.32	85.9±3.53
23	7.5	Gelatin 1	1:4	5 IPM	85.1	17.51±2.83	87.55±3.5
24	7.5	Gelatin 1	1:4	10 PEG	80	19±1.22	95±3.6
25	7.5	SDS 1	1:2.5	-	24.5	23.31±2.96	81.6±3.08

[a] calculated as the average value ± SD; [b] EE% means encapsulation efficiency percent

Table 2: Formulation Parameters and Properties of MX Loaded PCL, and PCL- PLGA Blend Microsphere formulae

F. No	PCL %w/v	emulsifier %w/v	D:P ratio	additives (%w/w)	Yield %	% content ± SD	EE% ± SD
26	7.5	Gelatin 2	1:2.5	-	65.04	27.23 ± 0.45	95.3 ± 1.83
27	7.5	Gelatin 2	1:3	-	61.19	23.18 ± 2.83	92.72 ± 5.4
28	7.5	Gelatin 2	1:4	-	58.32	16.37 ± 0.46	81.8 ± 2.19
29	7.5	Gelatin 2	1:2.5	2.5 IPM	80.9	27.16 ± 0.6	95 ± 2.08
30	7.5	Gelatin 2	1:2.5	5 IPM	70.4	28 ± 1.08	96.2 ± 2.89
31	7.5	Gelatin 2	1:2.5	10 IPM	68.8	26.48 ± 2.03	94.68 ± 7.14
32	7.5	Gelatin 3	1:2.5	-	71.4	27.28 ± 0.59	95.48 ± 5.02
33	7.5	Gelatin 4	1:2.5	-	80.3	26.22 ± 1.43	91.7 ± 0.63
34	7.5	MC 2	1:2.5	-	68.1	26.73 ± 0.72	88.55 ± 2.49
35	7.5	PVA 2	1:2.5	-	69.3	25.21 ± 0.95	93.21 ± 3.28
36	10	Gelatin 2	1:2.5	-	67.7	28.1 ± 0.62	98.35 ± 1.65
37	12.5	Gelatin 2	1:2.5	-	72.57	28.4 ± 0.48	99.4 ± 1.21
38	7.5	Gelatin 2	1:2.5	10 PEG	66.6	26.08 ± 1.56	91.28 ± 5.44
39	7.5	Gelatin 2	1:2.5	20 PEG	71.4	25.26 ± 0.6	88.41 ± 4.35
40	7.5	Gelatin 2	1:2.5	30 PEG	52.3	24.84 ± 0.12	86.94 ± 3.95
PCL-PLGA blends							
F. No	**Polymer %w/v**	**PCL % W/V**	**emulsifier %w/v**	**D:P ratio**	**Yield %**	**% content ± SD**	**EE% ± SD**
41	7.5	20%	Gelatine 2	1:2.5	72.3	26.67±2.2	93.34±7.68
42	7.5	40%	Gelatine 2	1:2.5	66.6	23.17±0.08	81.1±.3
43	7.5	60%	Gelatine 2	1:2.5	67.2	22.74±0.42	79.6±1.5

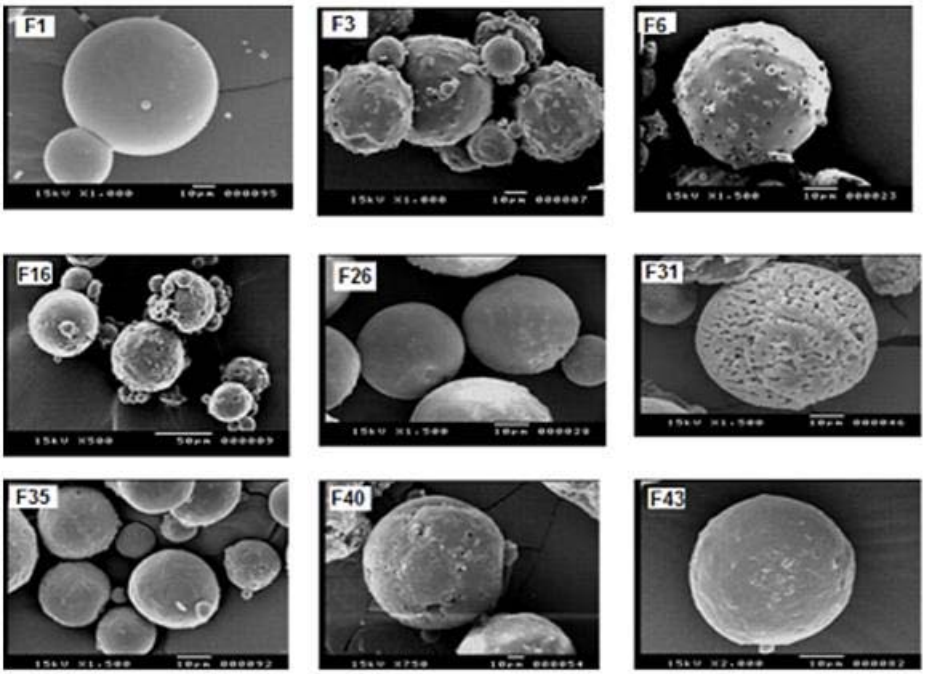

Figure 2: SEM images of microspheres formulations reveal change in surface characteristics with the use of different formulation additives.

Microspheres Particle Size

Microspheres size results showed that there was an increase in mean particle sizes for formulae F1, F2 and F3, with drug: PLGA ratios of 1:4, 1:3 and 1:2.5, respectively. In addition, the increase in drug: PCL ratio resulted in increase in the mean size of the produced microspheres formulae F28, F27 and F26 for drug: PCL ratios 1:4, 1:3 and 1:2.5, respectively. Upon using a blend of PCL and PLGA, PCL: PLGA 1:4 and 3:2, to prepare F41 and F43, respectively, a decrease in the mean diameter of the prepared microspheres was noticed, as PCL: PLGA ratio increases. On the other hand, the mean particle size of the PLGA microspheres was found to decrease from 41.57 to 26.12 µm by the increase in gelatine concentration from 1% w/v (F6) to 3 %w/v (F8). For PCL polymer, microspheres showed a decrease in size from 27.98 to 23.94 µm upon increasing gelatine concentration from 2% w/v to 4 %w/v for F26 and F33, respectively. MC microspheres (F34) were smaller in size compared with microspheres prepared using PVA (F35). Span values results of the prepared microspheres range from 0.9 to 1.27 that indicate uniform size microspheres.

Encapsulation Efficiency

Results in Tables 1 and 2 revealed that increasing the drug: polymer ratio from 1:4 to 1:2.5 (F1, F2 & F3 for PLGA and F28, F27 & F26 for PCL) increased the EE%. The influence of emulsifier type was investigated using 1% of four different emulsifiers, Gelatine, MC, PVA and SDS for formulae F6, F9, F10 and F11, for PLGA respectively. Table 1 reveals that the increase in emulsifier concentration resulted in reduction in the EE% values. In absence of additive, the increase in the emulsifier concentration showed no significant effect on EE% (Formulae F3, F17 and F18). Regarding the influence of additives, IPM and PEG 6000 were used as channelling agents to improve drug release from the prepared microspheres [13]. The EE% of the MX-loaded microspheres was not significantly affected by the incorporation of IPM and PEG 6000 (Table 1). Previous report results showed no relation between IPM concentration and drug-loading efficiency [14].

Drug Release Study

The reduction in the mean size as a result of changing the emulsifier type (gelatine, MC, PVA and SDS) for PLGA microspheres showed an influence on the in vitro drug release. The data obtained from EE% were correlated well with those obtained from the release experiment, i.e. smaller particles (F9) released 90.63% of the encapsulated drug during the first day, and 97.86% at the end of the experiment, compared with 27.32% and 50.1% for larger microspheres (F6). For PCL microspheres, upon use of different emulsion stabilizers other than gelatine viz., PVA and MC (2 % w/v of each), the release pattern curves were similar in shape. Small microspheres, with higher surface area, usually have higher release rates with significant initial burst compared to large particles [15, 16]. Variation of emulsifier concentration affected the release patterns of the prepared formulae. For PLGA microspheres, three concentrations of gelatine were used, 1%, 2% and 3%. Smaller particles (F8) released 55.7% of the encapsulated drug during the first day, and 87.8% at day 4, compared to 27.3% and 51% for larger microspheres (F6). For PCL microspheres, the increase of gelatine concentration from 2% to 3 then to 4%w/w (F26, F32 and F33, respectively), showed 100% drug release after 3 days for formulaeF32 and F33. While, F26 reached 100% drug release after 5 days with minimal burst of 33% after 3 hours from the start of the experiment.

The effect of polymer concentration on the release pattern was investigated by keeping all other parameters unchanged. The Released amounts of MX from three formulae F13, F12, and F6 was slowed by increasing the PLGA concentration during the first 10 days of the experiment (Fig. 3A). For PCL polymer, results showed that decrease in both drug release rates and burst amounts as a result of increase in PCL concentration (Fig. 3B). Two types of additives were used, hydrophilic (PEG 6000) and hydrophobic (IPM) in order to modulate the release patterns. The results showed direct relation of the concentration of the channelling agent used with the cumulative amounts of drug released (Fig.3 C -F).

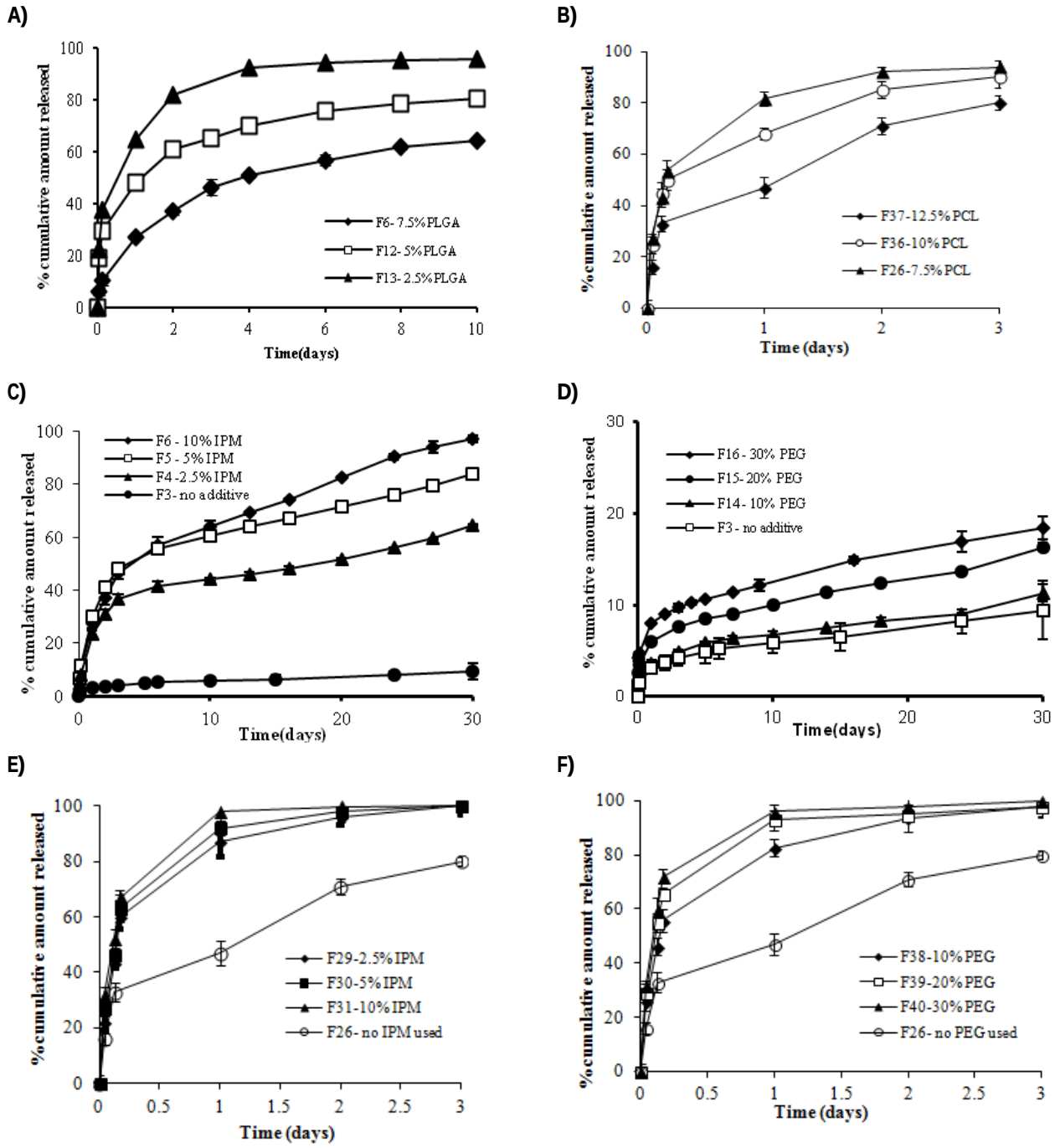

Figure 3: Effect of polymer concentration (A & B) and additives, IPM & PEG, concentration (C-F) on the Release characteristics of the prepared microspheres. A- PLGA, B- PCL, C, PLGA microspheres with IPM, D) PLGA microspheres with PEG, E) PCL microspheres with IPM and F) PCL microspheres with PEG.

Different proportions of PCL were added to PLGA for the preparation of microspheres, namely 20%, 40% and 60%w/w to form F41, F42 and F43, respectively as shown in Table 2. The use of polymer blend in F41, F42 and F43 has markedly improved the release pattern of MX. In addition, drug release behaviour showed a more sustained pattern, with lower initial burst compared with formulae prepared by PLGA only (F3) and PCL only (F26)

(Fig. 4). Formula F 41 showed the optimum initial burst release with highest yield%, % content and EE% compared with F42 and F43. Accordingly, formula f41 was selected for the in vivo studies.

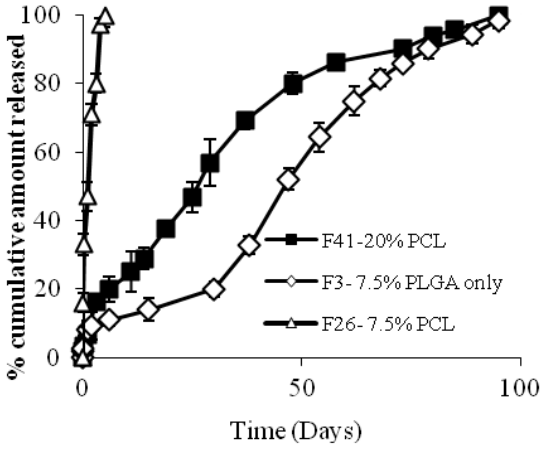

Figure 4: Influence of polymer blend on the release characteristics of the prepared microspheres

Pharmacokinetic Study

Plasma drug concentrations following various times of the intramascular injection of 30 mg of MX as microspheres (F41) or suspension were used in the pharmacokinetic study. MX plasma data after IM injection were fit to a one-compartment model with first-order absorption and elimination (Table 3) according to the format (equation 3):

$$C = A\left(e^{-k_{el}t} - e^{-k_a t}\right) \qquad (3)$$

Where: C is the concentration of MX in plasma at time t, A: constant coefficient or intercept and k_{el} and k_a are the rate constants of elimination and absorption, respectively. Data fitting required the incorporation of lag time.

Table 3: Pharmacokinetic parameters of MX in plasma of rats after single IM injection of 30 mg MX from microspheres (F41) and suspension.

Pharmacokinetic parameter[a]	Microspheres	Suspension
C_{max} (µg ml^{-1})	20.095	277.322
T_{max} (h)	23.22	2.732
K_a (h^{-1})	0.128	0.943
$t_{1/2}$ ka (h)	5.73	0.734
K_{el} (h^{-1})	0.0146	0.0155
$t_{1/2}$ (h)	47.47	44.7
AUC (µg h ml^{-1})	2368.3	3868.22
T_{lag} (h)	0.16	0.11

[a] Pharmacokinetic parameters were assessed by fitting individual concentration-time data to a one-compartment model. C_{max}, maximum concentration; T_{max}, time of maximum concentration achieved after administration; K_a, absorption rate constant; $t_{1/2Ka}$, absorption half-life; K_{el}, elimination rate constant; $t_{1/2}$, elimination half-life and AUC, area under the MX concentration-time curve .

DISCUSSION

The influence of different formulation parameters along with the effect of hydrophilic and lipophilic additives was investigated. In addition, attempts were made to extend the release time of MX-loaded microspheres by the use of polymer blending. PCL can be blended with other polymers including PLGA to manipulate the rate of drug release [10]. SEM results revealed the formation of porous surface microspheres by the addition of channelling agent, which could be related to a considerable amount of channelling agent dissolved in DCM that was extracted out of the formed system. The formed pores and channels allow the drug diffuse out to the aqueous phase. In general, microspheres formulated by using PCL or PCL-PLGA blend were less smooth than those prepared by using PLGA polymer. This could be attributed to the use of quench water which accelerated the rate of DCM removal from the microsphere matrix [17].

Polymer concentration in the organic phase showed a significant effect on the particle size of the microspheres. It is assumed that the viscosity of the oily phase increased by the increase in polymer concentration, which exerted resistance in the emulsification process against the rotation of the paddle, forming larger particles [18]. The denser microspheres matrix formed upon using higher polymer concentrations may hamper the drug diffusion out of the microspheres, as a result of the increased viscosity of the organic phase, during solvent evaporation and washing steps [19,20]. In addition, the mean microsphere sizes increased in a direct proportion to the drug: polymer (PLGA or PCL) ratio. This could be attributed to the increased frictional force of the internal phase upon increasing drug concentration [21]. Similar findings were obtained by Elkheshen et al., when microencapsulated metoclopramide in PLGA [22] and Kiliçarslan and Baykara formulated Verapamil loaded Eudragit microspheres [23]. In addition, the increased EE% of MX by the increase in drug: polymer ratio is attributed to the retention of drug in the organic phase as the microspheres solidify [24]. This increase in EE% could also be as a result of the increase in the amount of the added drug, to keep drug: polymer ratio unaffected.

The increase in emulsifier concentration is believed to prevent the fusion of small droplets into a large one, which generates small sized microspheres [25]. It is obvious that enhancement of EE% can be correlated with the increase in size of the microspheres upon using lower concentrations of emulsifier, as the drug is dispersed in the organic phase as suspension. This suggests that as the average size of the microspheres becomes smaller, the size range of drug crystals suitable to be encapsulated becomes narrower that affects EE%. Wider size ranges, and hence, more drug crystals can be encapsulated as the average size of the microspheres increases, resulting in improved EE%.

Optimization of the release properties of the prepared microspheres took place through the variation of formulation factors, as PCL concentration, emulsifier type and concentration, and incorporation of additives. The retardation of release pattern with the increase in polymer concentration is attributed to the increase in microspheres mean size. In addition, at higher PLGA concentrations, release retardation may also be attributed to the formation of a denser microsphere matrix internal structure [26]. Variation of PCL concentration in the organic phase alters the release pattern, as it affects the density of the PCL matrix and the size of the produced microspheres [27, 28]. The increase in the concentration of the polymer in the organic phase was believed to slow down the rate of drug diffusion, as a result of the increase in the matrix density of the microspheres [26, 29]. Moreover, it would be harder for the drug to migrate through the dense matrix to the surface that also reduces the burst release. Accordingly, drug deposition onto or near the surface is expected to be lower in high-PCL concentration formulae than that in those with lower concentrations. For these reasons, the decrease in both drug release rates and the burst amounts, due to increasing PCL concentration, can be justified.

Release patterns are affected by the variation of emulsifier concentration as it affects particle size, which in turn affects EE%. Increase in the emulsifier concentration is believed to reduce the interfacial tension, consequently emulsion globules break up, yielding smaller microspheres.Incorporation of channeling agent, IPM or PEG 6000, changed the structure of microsphere matrix as a result of leaching out of the channelling during microsphere preparation to form porous matrix with channels that provided better access to the release medium through which drug could diffuse[30, 31]

PCL is used in combination with various polymers for prpeparation of microspheres to modify the rate of drug release [32]. Polymers are frequently used in combinations for developing sustained release dosage forms with improved performance compared with the individual polymer [33]. Increasing the percentage of PCL used in the formulation from 20% to 40% then to 60%w/w resulted in a decrease in the % yield. This can be explained by the increased degree of adherence of polymer-drug moist paste around the metallic paddle of the mechanical stirrer, when increasing the used percentage of PCL. The reduction in EE% (Table 2) is attributed the decrease in mean particle size.

In vivo parenteral pharmacokinetic results revealed that MX suspension produces a depot effect as a result of the low aqueous solubility of the drug. It is noticed that the suspension produces high plasma concentration of the drug over a period of two days after injection, and the therapeutic plasma levels of the drug (0.5-1.5 µg/ml and up to 25 µg/ml) [34] were achieved between the third and the 14th day of the experiment. This period of 12 days of therapeutic levels compared to 21 days achieved for the injected microspheres. The dump effect was not observed upon using microspheres as a result of the slow drug release from the polymeric matrix of the microspheres. The suspension shows higher C_{max} and shorter T_{max} accompanied by a higher K_a when it was compared with microspheres Table 3. However, it is important to notice that the suspension gave almost the same value of K_{el} of microspheres as K_{el} is independent on the dosage form of the drug. The results have also revealed that the suspension showed a high bioavailability compared to microspheres. The relative bioavailability of microspheres (relative to suspension) calculated on the basis of AUC was found to be 0.6. This may be as a result of retaining some drug in the polymeric skeleton of microspheres (by adsorption or other mechanism).

CONCLUSION

MX-loaded biodegradable microspheres could be successfully prepared by using O/W ESE method with improved yield and encapsulation efficiency values. PLGA-PCL blend Microspheres showed improved sustained release pattern with lower initial burst. In Vivo studies in rats showed that injection of formula F41 microspheres achieved therapeutic MX levels for 21 successive days.

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