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ORIGINAL ARTICLE

Impact of microparticle formulation approaches on drug burst release: a level A IVIVC

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Abstract

Aim: To study the effect of poly(D,L-lactic-co-glycolic acid) (PLGA) microparticles (MPs) preparation techniques on particle physical characterization with special emphasis on burst drug release. *Methods*: A basic drug clozapine was used in combination with acid-terminated PLGA. Two approaches for MP preparation were compared; the *in situ* forming microparticle (ISM) and the emulsion-solvent evaporation (ESE) methods using an experimental design. The MPs obtained were compared according to their physical characterization, burst release and $T_{80\%}$. An *in vivo* pharmacokinetic study with *in vitro–in vivo* correlation (IVIVC) was also performed for the selected formula. *Results*: Both methods were able to sustain drug release for three weeks. ISM produced more porous particles and was not effective as ESE for controlling burst release. A good IVIVC ($R^2 = 0.9755$) was attained when injecting the selected formula into rats. *Conclusion*: MPs prepared with ESE showed a minimum burst release and a level A IVIVC was obtained when administered to rats.

Keywords

Burst release, factorial design, *in situ* forming microparticles, IVIVC, polymer *Tg*, uncapped PLGA

History

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Introduction

Poly(D,L-lactic-co-glycolic acid) (PLGA)-based microparticle (MP) systems are largely exploited in controlled drug delivery. They have the advantage of supplying a continuous amount of drug, but are known to suffer from burst release effect.

Burst release from monolithic delivery systems has been ascribed to a diversity of factors, mainly physical, due to drug surface adsorption and the porous structure of the delivery system, chemical, relying on polymer–drug interactions, in addition to the formulation process conditions (Huang and Brazel, 2001; Fu et al., 2003; Mao et al., 2007).

The accumulation of a portion of drug load on the polymer matrix surface during preparation is attributed to drug diffusion and migration by convection with the polymer solvent during polymer solidification. This results in drug heterogeneous distribution within the matrix, with higher concentrations at the surface, leading to burst release (Kishida et al., 1998). Burst release had been also correlated with the rate of polymer hardening (Luan and Bodmeier, 2006b).

Another explanation resides in the presence of two distinct pools of drug loaded inside the delivery system: a pool of movable drug diffusing freely through pores upon hydration of the matrix, and an immobilized drug portion, which will diffuse only after hydrolytic degradation of the matrix. This is theoretically similar to percolation, where solute molecules connected by pores may diffuse, while sequestered solute is confined in the matrix (Tzafriri, 2000).

The burst release drawback has been treated with different additives incorporated in microparticulate systems such as fluorosilicone oil with ganciclovir (Herrero-Vanrell et al., 2000), phosphatidylcholine with albumin (Chung et al., 2006) and rat serum albumin combined with $NaHCO_3$ in case of lysozyme (Srinivasan et al., 2005). Labrafil®, a polyethylene glycol 300 (PEG) derivative (polyoxyethylated oleic glyceride), had been used to control the release rate of indomethacin and ibuprofen from PLGA MPs for intra-articular administration (Fernández-Carballido et al., 2004; Puebla et al., 2005). However, when used with acyclovir for intraocular delivery, an opposite release behaviour was obtained (Martínez-Sancho et al., 2003). Although the large number of additives exploited in research to control burst release from PLGA MPs, none of these studies encompass their *in vivo* performance.

MPs have been prepared by several methods including solvent evaporation, organic phase separation, spray-drying or supercritical fluid technology. Furthermore, injectable liquids containing drug and polymer capable of forming MPs after injection into the body had been tackled and known as *in situ* forming microparticle (ISM): a water-miscible solvent containing both drug and polymer is emulsified into a USP-approved parenteral solvent such as peanut oil or sesame oil. Upon injection, the water-miscible solvent, e.g. dimethyl sulfoxide, N-methyl-2-pyrrolidone (NMP) or 2-pyrrolidone, diffuses to the aqueous environment (phosphate buffer *in vitro* or tissue fluid *in vivo*) forming the MPs (Bodmeier, 1997; Kranz et al., 2008). A slower diffusion depending on polymer molecular weight and oil concentration was found to control burst release effect. Previous works demonstrated a good *in vitro–in vivo* correlation (IVIVC) with a prolonged effect of

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bupivacaine hydrochloride and vinpocetine after intramuscular (IM) administration of ISM systems to rats (Kranz et al., 2008; Li et al., 2008).

Being a basic drug containing a secondary amine group, clozapine (CZP) is expected to interact electrostatically with the acid-terminated (uncapped) PLGA polymer, thus with the possibility of decreasing the burst release (Budhian et al., 2005).

The objective of this work was to study the effect of ISM and emulsion-solvent evaporation (ESE) on drug burst release. Complete MPs characterization obtained from the two methods was performed. An *in vivo* pharmacokinetic study and IVIVC after IM injection of the selected MP formulation in rats were established.

Materials and methods

Materials

CZP was kindly supplied by Apex Pharma (Cairo, Egypt); 50:50 uncapped poly(DL-lactide-co-glycolide) (DL-PLGA 5002 A and 5004 A), with molecular weights and inherent viscosities = 17 000 g/mole, 0.2 dL/g and 44 000 g/mole, 0.4 dL/g, respectively, were supplied by PURAC Biochem (Gorinchem, The Netherlands). Polyvinyl alcohol (PVA) Mw 14 000 was purchased from Laboratory Rasayan, SD Fine Chem Ltd. (Boisar, India). Labrafil® M 1944 CS [a mixture of mono-, di- and triglycerides and mono- and di-fatty esters of PEG, oleic acid being the predominant fatty acid] and glyceryl palmitostearate were supplied by Gattefossé (Saint-Priest, France). NMP, methanol (HPLC grade), ethyl acetate (HPLC grade), glacial acetic acid (HPLC grade) and triethylamine (HPLC grade) were purchased from Sigma-Aldrich Company (St. Louis, MO). Sesame oil was purchased from Henry Lamotte GmbH (Bremen, Germany). Pluronic F-68 was purchased from BASF AG (Ludwigshafen, Germany). Potassium dihydrogen phosphate, disodium hydrogen phosphate, methyl cellulose, Tween 80, dichloromethane (DCM) and methyl alcohol were purchased by El Nasr Pharmaceutical Chemicals (ADWIC) (Abou Zaabal, Cairo, Egypt). Celecoxib was kindly supplied by Sedico Co. (6th October City, Giza, Egypt). Sterile highly purified water for HPLC was purchased from Otsuka Co. (Cairo, Egypt).

Preparation of CZP-loaded MP systems

Preparation of CZP-loaded ISM

The ISM systems were prepared as previously described in reference (Kranz et al., 2008). The drug-containing polymer solutions (PLGA 5002 A or 5004 A) in NMP in addition to Pluronic F-68 1% (w/w) were emulsified into sesame oil containing glyceryl palmitostearate 2% (w/w) by probe sonication (Microson, Stewartstown, PA) for 1 min under ice cooling to minimize the heat generated from the sonicator energy resulting in thermal damage (Zuccheri and Asproulis, 2012). The prepared emulsions were then injected directly in the dissolution medium where MPs were formed.

After conducting preliminary experiments, a general full factorial design experiment was built up to study the effect of three factors, namely: PLGA type (X_1), at two levels (5002 A and 5004 A), each type at three concentration (X_2) levels (10, 20 and 30%) and the polymer to oil ratio (X_3) at two levels (1:1 and 1:2), leading to $3 \times 2^2 = 12$ formulae. The responses studied were Y_1: the initial burst release at the first day and Y_2: the time required for 80% CZP release ($T_{80\%}$). The assigned number for each formula and the typical design of the factorial experiment are shown in Table 1.

Table 1. The formulae of CZP-loaded ISM used in the factorial design.

PLGA type	PLGA concentration in NMP (%w/w)	Polymer phase to oil phase ratio 1:1	1:2
PLGA 5002 A	10	1a	7a
	20	2a	8a
	30	3a	9a
PLGA 5004 A	10	4a	10a
	20	5a	11a
	30	6a	12a

Table 2. The formulae of CZP-loaded PLGA 5004A MPs used in the factorial design.

PLGA concentration (%w/v) in organic phase	Labrafil concentration (%w/w) of polymer weight 0	10	30
4	1b	4b	7b
8	2b	5b	8b
12	3b	6b	9b

Preparation of CZP-loaded PLGA MPs

PLGA MPs loaded with CZP were prepared by an oil-in-water (o/w) ESE technique (Sinha and Trehan, 2008). The oil phase consisted of DCM in which the required amounts of drug and PLGA 5004 A, with and without Labrafil, were dissolved, added to PVA solution and stirred using a propeller stirrer (Heidolph Elektro KG, Kelheim, Germany) at 1000 rpm for 4 h. The resulting MPs were harvested by filtration using Whatman No. 5 filter paper (Whatman, Inc, Florham Park, NJ), washed three times with distilled water and dried in a desiccator at room temperature for two days.

After conducting preliminary experiments, a general full factorial design experiment was constructed to study the effect of two factors: PLGA 5004 A (X_1) and Labrafil concentration (X_2) each at three levels (4, 8 and 12%) and (0, 10 and 30%), respectively, leading to $3^2 = 9$ formulae. The responses studied were Y_1: the drug incorporation efficiency (IEF), Y_2: the initial burst release at the first day and Y_3: the time required for 80% CZP release ($T_{80\%}$).

The factorial experiment design with the consigned number for each formula is represented in Table 2.

Determination of IEF of CZP in PLGA MPs

Encapsulated CZP was determined by dissolving the dried MPs (10 mg) in 100 ml DCM. The CZP content in the PLGA MPs was determined spectrophotometrically (Shimadzu UV visible 1601 PC, Kyoto, Japan) at λ_{max} 297 nm. After conducting control experiments, the components of the MPs did not interfere with CZP at this wavelength.

The IEF of CZP was calculated according to the following equation:

$$IEF = D_m \times 100/D_t \quad (1)$$

where Dt is the theoretical amount of CZP loaded to PLGA solution and Dm is the actual amount of CZP in the prepared MPs (Zidan et al., 2006). Experiments were run in triplicate.

In vitro CZP release studies from ISM and MPs

An accurately weighed amount of the prepared ISM emulsion or dried PLGA MPs, containing 2 mg CZP, were placed into dialysis

bags (Mw cut off 12 000–14 000 Da) containing phosphate buffer pH 7.4 (D'Souza S and DeLuca, 2006). The bags were placed into 50 ml phosphate buffer, pH 7.4, containing 25% (v/v) methanol to achieve the sink conditions, on a magnetic stirrer adjusted at $37 \pm 0.5\,^{\circ}C$. At predetermined time intervals, 2 ml samples (which were replaced with fresh medium) were withdrawn and assayed UV-spectrophotometrically at 293 nm. The *in vitro* release studies were performed in triplicate for each formula.

Mechanism and mathematical modelling

In order to determine the mechanism of drug release, the data were analyzed on the basis of zero-order, first-order and Higuchi model (Higuchi, 1963; Hadjiioannou et al., 1993; Bourne, 2002).

$$\text{Zero-order equation} : C_t = C_0 - k_0 t \quad (2)$$

$$\text{First-order equation} : \log C = \log C_0 - (k_1/2.303)t \quad (3)$$

$$\text{Higuchi equation} : C_t = k_2 t^{1/2} \quad (4)$$

Where C_t is the cumulative percentage of drug released at time t; C is the cumulative remaining drug percentage at time t; C_0 is the drug initial concentration; and k_0, k_1 and k_2 are the release rate constants for the zero-order, first-order and Higuchi release model, respectively.

The Korsmeyer–Peppas model (Equation (5)), a semi-empirical model, correlating drug release to time by a simple exponential equation for the first 60% drug release, has been also used to evaluate drug release (Korsmeyer et al., 1983; Costa and Lobo, 2001).

$$M_t/M_\infty = k \cdot t^n \quad (5)$$

M_t/M_∞ is the proportion of drug released at time t, k is the kinetic constant and the exponent n has been proposed as indicative of the release mechanism. In this context, $n \leq 0.43$ indicates Fickian release and $n = 0.85$ indicates a Case II transport corresponding to purely relaxation controlled delivery. Intermediate values $0.43 < n < 0.85$ indicate an anomalous behaviour (non-Fickian) corresponding to coupled diffusion/polymer relaxation (Ritger and Peppas, 1987a,b).

Characterization of the hardened ISM and prepared MPs

Scanning electron microscopy

The ISM emulsion was injected into phosphate buffer (pH 7.4, 0.1% Tween 80) under stirring. After 5 h stirring, the formed MPs were filtered and dried in a desiccator for 48 h. The surface morphology of the hardened MPs and dried PLGA MPs was determined by scanning electron microscopy (SEM; Jeol Scanning Microscope, JSM-5500 LV, Tokyo, Japan). No chemical fixation or freeze drying methods were used in the preparation of samples for SEM. The dried MPs were mounted on round brass stubs and gold sputter-coated using SPI-Module gold sputter coater for 70 s under an argon atmosphere and then observed with a scanning electron microscope.

Particle size measurement

The mean diameter was quantitatively determined by measuring 100 MPs from the SEM micrographs using the Olympus Soft Imaging Solutions GmbH software (Münster, Germany). The scale bar for each micrograph was introduced into the software, and the MPs edges were indicated by the software. The number of MPs (100) measured for each population was sufficient to provide an accurate mean diameter (Berchane et al., 2007; Berchane et al., 2010).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed on a TA Instruments DSC-60 (Shimadzu; connected with thermal analyzer TA-501 and DeskJet 500 c printer) with liquid nitrogen cooling system. Accurately weighed samples (~2–5 mg) were hermetically sealed in aluminium pan. To determine the drug endotherm, heating was performed from 25 °C to 220 °C at a rate of 5 °C/min under nitrogen flow. For glass transition temperature (*Tg*) determination, the DSC analysis was done with an initial heating scan followed by a rapid quench cooling scan from 220 °C to −50 °C at a rate of 35 °C/min, in order to erase the polymer thermo-mechanical history, then the *Tg* was determined by a second heating scan from −50 °C to 100 °C at a rate of 5 °C/min (Bouissou et al., 2006; Yang et al., 2009).

In vivo studies

Preparation for in vivo *studies*

Sterilization by gamma irradiation. The selected formula was gamma irradiated by packing it in dry ice inside polyurethane container and then sterilized (Martínez-Sancho et al., 2004; Kamel et al., 2009). The irradiation facility used was cobalt-60 Gamma Chamber 4000-A. The received dose was 25 KGy, considered as adequate for purpose of sterilizing pharmaceutical products when the bioburden is not known (European Pharmacopoeia, sixth edition, 2006). The sterilized MPs were evaluated for their IEF compared to the non-sterilized MPs. The relevancy of the data obtained by UV spectrophotometry was tested by HPLC according to the assay (Manjunath and Venkateswarlu, 2005) described under section 2.10.3 and proved to be suitable for measurement of IEF after sterilization. CZP release profiles of sterilized and non-sterilized MPs were also compared using the difference factor (f_1) and the similarity factor (f_2) and were defined by the following equations (Moore and Flanner, 1996):

$$f_1 = \left\{ \left[\Sigma_t =_1^n |R_t - T_t| \right] / \left[\Sigma_t =_1^n R_t \right] \right\} \times 100 \quad (6)$$

$$f_2 = 50 \times \log \left\{ \left[1 + (1/n) \Sigma_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (7)$$

where *n* is the number of time points, R_t and T_t are the cumulative percentage drug dissolved at each of the selected n time points of the reference and test product, respectively. In order to consider similar dissolution profiles, f_1 values should be lower than 15 (0–15) and f_2 values should be higher than 50 (50–100) (Costa and Lobo, 2001).

Injectability test. The test was performed using a device developed and validated in our laboratory after (Holayel, 1996; El Zaafarany et al., 2010). The selected formula (10 mg) was suspended in 1 ml of 1.5% methylcellulose and 0.9% NaCl solution and placed in 3-ml plastic syringes. The syringe was then vertically clamped and the probe of the device lowered until initial contact with the plunger of the syringe was observed. Constant forces were applied in multiples of 1 N. Maximum force required to expel the suspension through a 23G needle was determined.

In vivo *experiment*

Intravenous and IM administration of CZP to rats. Animal experiments were conducted according to protocols approved by the Experiments and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University on the use of the animals. Male albino rats (200 ± 20 g) were used in

intravenous (IV) and IM administrations. Feeding and drinking were allowed freely during the whole study.

The CZP solution (4 mg/ml) was prepared by dissolving CZP in 0.25 N HCl adjusted to pH 5 with 1 N NaOH. The CZP suspension (4 mg/ml) and CZP-loaded MPs (F-9b) suspension (10 mg CZP/ml) was prepared by dispersing the drug or MPs, respectively, in sterile water containing 1.5% methylcellulose and 0.9% NaCl.

Eighteen white male albino rats were divided into three groups (six for each group) and used for the pharmacokinetic study. The treatment was performed as follows:

Group I received CZP solution, group II received CZP suspension and group III received CZP-loaded MPs (F-9b).

Rats were injected IV with a single dose of CZP solution (10 mg/kg) for group I (Manjunath and Venkateswarlu, 2005), IM with CZP suspension (20 mg/kg) for group II (Bolden-Watson et al., 1993) and CZP-loaded MPs (100 mg/kg) for group III. The single dose of an extended release preparation is commonly higher than that of an immediate release one and should be administered at doses that are at least 10 times the IV dose for the free drug (Krugner-Higby et al., 2009).

Sampling. Samples of 0.5 ml blood were then withdrawn from the retro-orbital venous plexus puncture at different time intervals of 0.25, 0.5, 0.75, 1, 2, 4, 6 and 12 h for CZP solution, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h for CZP suspension and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 312, 360, 408, 456 and 504 h for CZP-loaded MPs after drug administration. Samples were collected in sterile tubes containing EDTA-K3 and centrifuged at 3500 rpm for 15 min. The separated plasma was transferred into Eppendorfs, closed and stored at −20 °C until assayed.

Plasma sample analysis. The method involves the extraction of CZP with ethyl acetate in the presence of celecoxib as an internal standard (Manjunath and Venkateswarlu, 2005). Plasma volume of 200 µl of was pippetted into a glass centrifuge tube. The internal standard (600 ng/20 µl) and 200 µl of 2 M sodium hydroxide were added and mixed well. The extracting solvent were added and vortexed for 5 min followed by centrifugation at 3500 rpm for 15 min. The organic phase was separated and evaporated under reduced pressure. The residue was reconstituted in 400 µl of mobile phase and 20 µl were injected into HPLC column. The ratios of peak areas of drug to internal standard were calculated.

The chromatographic system consisted of Agilent Technologies (Santa Clara, CA) 1200 series LC – G 1311 A solvent delivery pump and G1315D diode array detector. The column used was Agilent TC-C_{18} analytical column (5 µm particle size; 250 × 4.6 mm ID). The mobile phase consisted of methanol–water–triethylamine (75:25:0.5, v/v/v). The UV detector was set at 254 nm, and the sensitivity was set at 0.001 absorbance units full scale. The flow rate was 1 ml/min. The data was recorded and calculated using ChemStation B.04.01 software (Santa Clara, CA).

Pharmacokinetic analysis. The mean plasma concentration–time data after IV administration of the CZP solution was used to obtain the fitted compartmental model by R Foundation for Statistical Computing ISBN 3-900051-07-0 (R Development Core team, Vienna, Austria) program. The terminal elimination rate constant (*K*e) was estimated using the values least-squares regression in the terminal log-linear region of plasma concentration–time curve. The terminal elimination half-life ($T_{1/2}$) was also calculated as 0.693/*K*e.

The plasma drug concentration versus time data after IM administration of CZP suspension and CZP-loaded MPs in individual rats were analyzed by non-compartmental estimations using Thermo kinetica software (version 5.0.11, Philadelphia, PA). Maximum serum concentration (C_{max}) and the time to reach C_{max} (T_{max}) were recorded.

The areas under the curve from time zero to last sampling time (AUC_{0-t}) and the areas under the first moment curve (AUMC) were determined by the linear trapezoidal rule. The areas under the curve from time zero to infinity ($AUC_{0-\infty}$) were calculated as $AUC_{0-t} + C_t/K_e$, where C_t is the last detectable plasma concentration and t is the time at which this concentration occurred. Mean residence time was determined by dividing AUMC by AUC.

All data were expressed as mean ± standard error of the mean.

IVIVC. The recorded data from the pharmacokinetic study were used to develop the IVIVC (Level A). The relationship between percent *in vitro* drug released in phosphate buffer pH 7.4 and the fraction of drug absorbed *in vivo* (F_a) was examined. The F_a was calculated using the Wagner–Nelson method *via* the following equation (Wagner and Nelson, 1963):

$$F_a = (C_t/K_e + AUC_0{-}t)/AUC_0{-}\infty$$

The relationship between percent *in vitro* drug released in phosphate buffer pH 7.4 and percent AUC ($AUC_{0-t}/AUC_{0-\infty}$) was also examined (Chu et al., 2006). The values of correlation coefficient (R^2), slope and intercept were calculated from the linear regression analysis of the IVIVC plots.

Data analysis

The factorial design experiment data were evaluated statistically using MINITAB for Windows (release 15.1.30.0, 2007, State College, PA) computer program. The analysis of variance (ANOVA), the main effects and interactions were calculated. Before applying ANOVA, visual inspection of the residual values histogram of the specified response or the linearity of plots was checked for normal distribution of the residuals.

Results

Evaluation of data from the factorial design experiments

Table 3(a and b) lists the values of dependent variables (responses) for the evaluation of CZP-loaded ISM and MPs, respectively, according to the factorial design.

ISM

Burst release percent and $T_{80\%}$. The ANOVA test (Table 4a) shows that PLGA type, concentration and polymer to oil ratio (P/O ratio) have significant main effects on burst release percent and $T_{80\%}$ ($p < 0.0001$). Either substituting PLGA 5002 A with PLGA 5004 A or increasing polymer concentration significantly decreased the burst drug release and increased $T_{80\%}$ ($p < 0.0001$). The polymer to oil ratio had the lowest significant effect on both responses. Previous workers reported a significant reduction in percentage of drug initially released by varying in the polymer to oil ratios from (1:1) to (1:2) ($p < 0.0001$) (Luan and Bodmeier, 2006a).

By reviewing the sources of variation, for burst release percent, it is clear that all interactions between the studied variables were non-significant, thus, the main effect results can be conclusive. By studying the significant two- and three-way interactions for $T_{80\%}$, it followed that increasing the PLGA concentration and decreasing the polymer/oil ratio significantly delayed the mean $T_{80\%}$, especially with PLGA 5004 A ($p < 0.0001$) (figure not shown). So it can be concluded that

Table 3. Responses values for the evaluation of (a) CZP-loaded ISM and (b) CZP-loaded MPs according to factorial design experiment.

(a)

	Responses (mean ± SD)[a]	
Formula code	Burst release at the first day (%)	$T_{80\%}$ (days)
1a	71.71 ± 3.09	3.00 ± 0.08
2a	69.12 ± 3.43	4.01 ± 0.05
3a	50.22 ± 3.03	8.05 ± 0.13
4a	64.57 ± 2.16	3.99 ± 0.13
5a	51.78 ± 2.45	6.90 ± 0.14
6a	42.70 ± 0.18	11.90 ± 0.09
7a	66.35 ± 4.46	5.20 ± 0.06
8a	63.68 ± 3.87	5.70 ± 0.13
9a	42.09 ± 2.25	7.79 ± 0.09
10a	45.96 ± 3.54	6.40 ± 0.08
11a	42.04 ± 2.36	9.90 ± 0.06
12a	38.50 ± 1.61	23.60 ± 0.13

(b)

	Responses (mean ± SD)[a]		
Formula code	EEF (%)	Burst release at the first day (%)	$T_{80\%}$ (days)
1b	91.49 ± 3.44	63.40 ± 1.67	4.33 ± 0.48
2b	94.25 ± 0.40	50.05 ± 2.53	7.00 ± 0.33
3b	99.33 ± 0.95	42.45 ± 0.99	8.21 ± 0.21
4b	93.03 ± 0.03	36.15 ± 0.99	14.83 ± 0.14
5b	96.97 ± 2.21	26.30 ± 4.81	15.83 ± 0.64
6b	99.45 ± 0.78	25.73 ± 1.36	15.58 ± 0.78
7b	95.67 ± 3.23	33.67 ± 4.77	19.00 ± 0.13
8b	97.9 ± 3.64	25.41 ± 3.08	19.92 ± 0.62
9b	100 ± 0.00	22.81 ± 0.97	19.17 ± 0.70

Notes: SD: standard deviation.
[a]Average of three determinations.

ISM formulae 12a containing 30% PLGA 5004 A with P/O ratio (1:2) had the lowest percentage of burst release at the first day (38.50%) with a prolonged release for about three weeks. Hence PLGA 5004 A was tried using the solvent evaporation method.

MPs

IEF. The IEF of the prepared MPs was in the range of 91.49–100%. ANOVA test (Table 4b) shows that none of the studied factors have significant main effects concerning CZP IEF into PLGA MPs and that no significant interaction was observed ($p > 0.0001$).

Burst release and $T_{80\%}$ responses. The interaction between the positively charged drug and the acid terminated polymer could not control the burst release as seen in formulae free from Labrafil. Both PLGA and Labrafil® concentrations have significant main effects (Table 4b) on the burst release with a higher effect for Labrafil ($p < 0.0001$) and the interaction between the studied variables was non-significant. On the other hand, only Labrafil concentration has significant effect on $T_{80\%}$ CZP release ($p < 0.0001$), with the effect of PLGA concentration being non-significant. Increasing Labrafil concentration from 0% to 30% prolonged significantly $T_{80\%}$. The interaction between the studied variables was also non-significant (figure not shown).

From all previous results, it could be concluded that PLGA MPs (F-9b) containing 12% PLGA 5004 A with 30% Labrafil had the lowest percentage of burst release at the first day (22.81%), which showed extended drug release for three weeks.

Table 4. *F*-values for factorial design experiment responses of (a) CZP-loaded ISM and (b) CZP-loaded MPs.

(a)

	F-value[a]	
Source of variation	Burst drug release (%)	$T_{80\%}$
PLGA type	59.14[b]	149.11[b]
PLGA concentration	43.90[b]	337.25[b]
Polymer/oil phase ratio	26.03[b]	74.89[b]
PLGA type – PLGA concentration	5.78	47.39[b]
PLGA type – Polymer/oil phase ratio	1.82	40.20[b]
PLGA conc. – Polymer/pil phase ratio	1.09	161.05[b]
PLGA type – PLGA concentration – polymer/oil phase ratio	2.18	22.09[b]

(b)

	F-value[a]		
Source of variation	EEF	Burst drug release (%)	$T_{80\%}$
PLGA concentration	6.16	20.84[b]	9.55
Labrafil concentration	1.29	72.88[b]	505.86[b]
PLGA concentration – Labrafil Conc.	0.23	1.20	3.89

Notes: [a]*F*-value = between-group variance/within-group variance.
[b]Significant at $p < 0.0001$.

Mechanism and mathematical modelling for drug release

In case of ISM, the R^2 values ranged from 0.8809 to 0.9764 for zero-order, from 0.7544 to 0.9718 for first-order and from 0.9403 to 0.9977 for Higuchi model. The respective R^2 values for PLGA MPs were from 0.7814 to 0.8265, from 0.8450 to 0.9436 and from 0.9317 to 0.9866 for zero-order, first-order and Higuchi models, respectively. The latter results confirm a Higuchi drug release mechanism for both systems. Fitting the release data to Korsemeyer–Peppas equation, the *n* values were smaller than 0.43 (range: 0.14–0.21) for ISM and lied between 0.43 and 0.85 (range: 0.48–0.83) for MPs.

Characterization of the hardened ISM and prepared MPs

For clarification the reasons behind the effect of different polymers, additives and preparation methods on burst release and $T_{80\%}$ of CZP, the following tests were performed.

SEM and particle size measurement

The surface morphology of the hardened ISM was determined by SEM as shown in Figure 1. The SEM study demonstrated that the particles had spherical and uniform shapes. The mean diameter was in the range of 10–30 µm. By comparing Figure 1(a and b) with Figure 1(c and d), it is obvious that ISM MPs formed with PLGA 5004 A were less porous, more dense in structure than those prepared with PLGA 5002 A. It is also clear that higher concentration of the same polymer type gave less porous particles.

The surface morphology of the CZP-loaded PLGA MPs was shown in Figure 2. The particles had spherical and uniform shapes. The mean diameter was in the range of 45–65 µm. Morphologically, SEM revealed that PLGA MPs without Labrafil were spherical with smooth surfaces (Figure 2a), whereas PLGA/Labrafil MPs exhibited homogeneous shapes with small concavities on their surfaces (Figure 2b and c) regularly distributed throughout the surface.

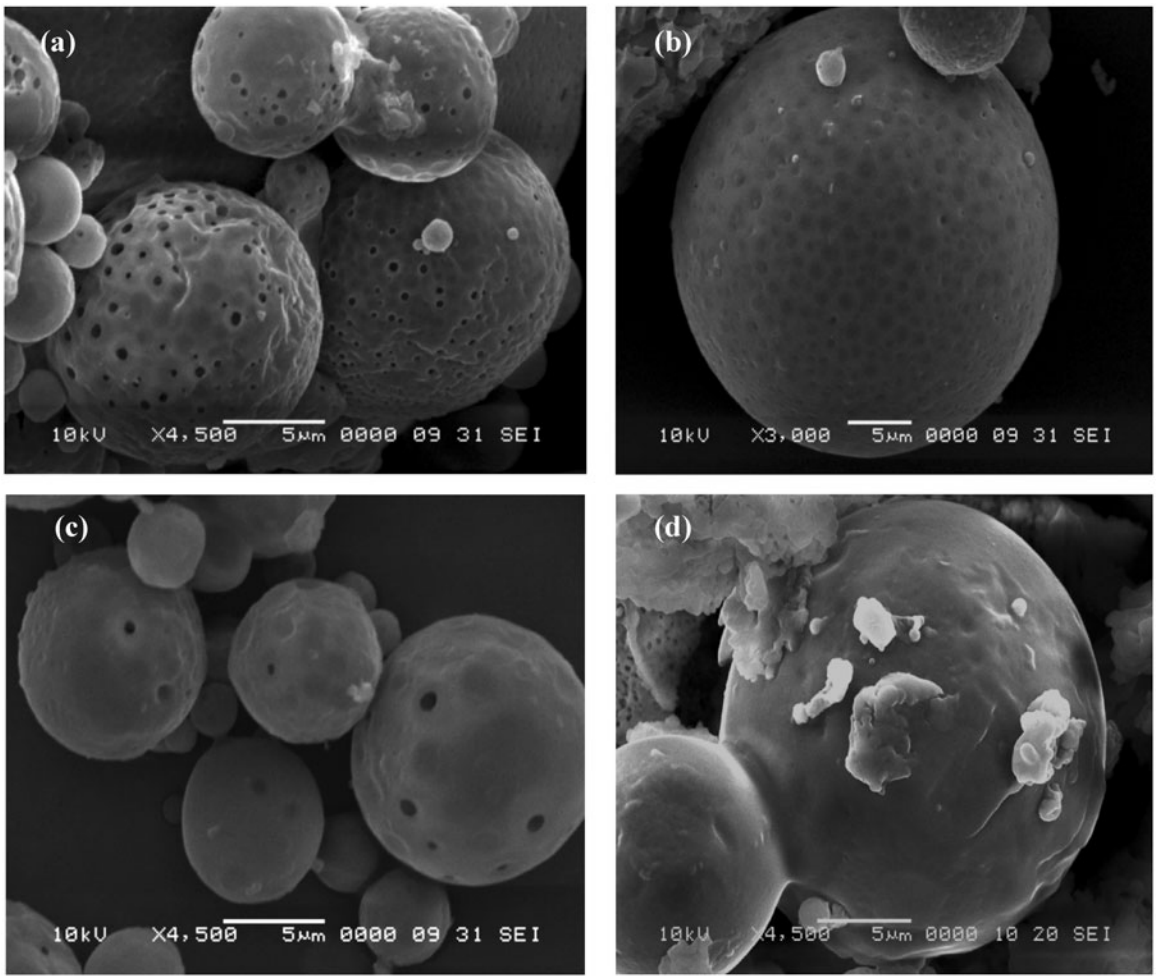

Figure 1. SEM pictures of CZP-loaded ISM prepared with 10% PLGA 5002 A (a), 30% PLGA 5002 A (b), 10% PLGA 5004 A (c) and 30% PLGA 5004 A (d).

Differential scanning calorimetry

Pure crystalline CZP showed a sharp endothermic melting peak at 183 °C (Figure not shown). Figure 3I(a) and Figure 3II(a) show the characteristic DSC thermographs of the second heating cycle where the *Tg* for pure PLGA 5002 A and 5004 A were observed at 41.84 and 50.31 °C, respectively. Sesame oil decreased their respective polymer *Tg* from 41.84 °C to 37.17 °C and from 50.31 °C to 46.19 °C [Figure 3I(b) and II(b)]. The inclusion of the basic drug CZP into PLGA 5002 A ISM showed a decrease in PLGA *Tg* from 37.17 to 31.96 °C [Figure 3I(c)] and an increase in polymer *Tg* from 46.19 to 50.51 °C in case of ISM prepared by PLGA 5004 A [Figure 3II(c)].

Figure 4(c) shows the characteristic DSC thermographs of the second heating cycle where *Tg* for unloaded PLGA MPs was observed at 50.35 °C. The addition of Labrafil MPs increased the polymer *Tg* from 50.35 °C to 57.14 °C (Figure 4a and c). Furthermore, and in contrast to ISM, the drug shows a plasticizing effect on this polymer where *Tg* decreased from 57.14 to 45.80 °C (Figure 4b).

In vivo studies

Preparation for in vivo *studies*

From the previous results, it can be concluded that MPs, containing 12% PLGA 5004 A and 30% Labrafil, showed the lowest burst release percent with three weeks release sustainment, hence, it was selected for further studies.

Sterilization by gamma irradiation. The IEF of the selected MPs formula (9b), after sterilization, was found to be $98.95 \pm 0.5\%$ compared to that of non-sterilized formula ($100 \pm 0\%$). Accordingly, sterilization with dry ice did not affect significantly the IEF% of the tested formula at $p < 0.01$ (Student's *t* test). It is evident that the burst release at the first day and $T_{80\%}$ for the sterilized formula did not differ significantly from that for the unsterilized one ($p < 0.01$). Furthermore, their release profiles were similar with f_1 value 3.088 and f_2 value 86.42.

Injectability test. The maximum force needed to inject 1 ml of MPs formula (9b) suspension prepared in a concentration of 10 mg/ml through a 23G needle was determined to be 10.80 ± 0.20 N using the method described under ''injectability test'' section.

Pharmacokinetic study

After single IV administration of the CZP solution at the dose of 10 mg/kg in rats, CZP was rapidly eliminated with K_e of $0.19 \pm 0.01\ h^{-1}$ and $T_{1/2}$ of 3.65 ± 0.15 h. The mean plasma concentration–time curves of CZP following IM injection of suspension and MPs at the dose of 20 and 100 mg/kg, respectively, in rats were shown in Figure 5.

Pharmacokinetics parameters of CZP after IM administration of suspension and MPs were shown in Table 5. The PLGA MPs sustained the release of CZP for 21 days after a short burst release. The drug absorbed *in vivo* calculated by the Wagner–Nelson

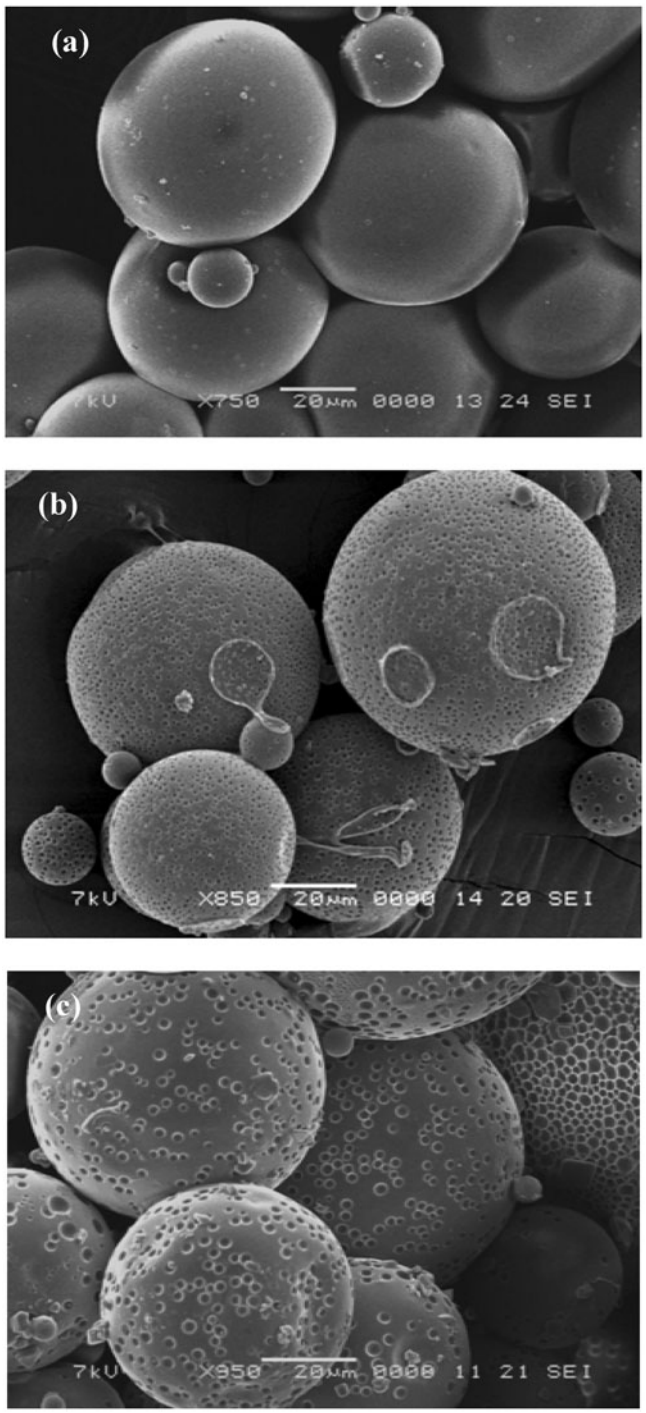

Figure 2. SEM pictures of CZP-loaded PLGA MPS (a), PLGA/10% Labrafil MPs (b) and PLGA/30% Labrafil MPs (c).

method showed that 100 ± 0.00 and $10.31 \pm 0.02\%$ of CZP was released within the first 24 h following IM administration of suspension and MPs, respectively. The CZP plasma concentration reached a C_{max} of 417.14 ± 83.90 ng/mL at the first hour for suspension and 355.84 ± 30.78 ng/mL for MPs after 48 h, respectively. The plasma drug concentration was completely depleted after day 2 for suspension and at day 21 for MPs with respective $AUC_{0-\infty}$ 6247.14 ± 468.34 and 157638.00 ± 10489.12 ng h/mL.

IVIVC

The percent *in vivo* drug absorbed and percent $AUC_{0-\infty}$ versus the amount of drug released *in vitro* plots were shown in Figure 6. A good linear regression relationship was observed between the percent *in vitro* dissolution in phosphate buffer pH 7.4 at 37 °C and the percent absorption ($R^2 = 0.9755$ with a slope $= 1.2589$ and an intercept $= -0.0849$, for the Wagner–Nelson method) and percent $AUC_{0-\infty}$ ($R^2 = 0.9705$ with a slope $= 1.2669$ and an intercept $= -0.0996$) after IM administration of the CZP-loaded MPs in rats.

Discussion

Independent variables and their levels were chosen based on preliminary studies where it appeared that generally, the polymer and oil concentrations significantly affected both the burst release percent at the first day and the time required for 80% CZP release (T80%) in case of ISM and MPs. The polymer type was also found to affect ISM release properties. Thus, a full factorial design experiment was built for both preparations. For ISM, three factors were studied; the PLGA type and concentration and the polymer to oil ratio, while for MPs, the independent variables were the polymer and oil concentrations. Built on the results of ISM, the type of polymer was excluded from MPs experiment. In case of ISM, the polymer concentrations were kept between 10 and 30%; as preliminary experiments revealed that polymer concentrations less than 10% failed to produce distinct MPs, following emulsion injection into the buffer, while polymer concentration higher than 30% formed non injectable highly viscous emulsion. In the same context, the polymer to oil ratio was kept at 1:1 and 1:2, otherwise the emulsion was not formed at higher ratio or difficult to inject at lower ratio. While in case of MPs, the polymer concentrations were kept between 4 and 12%, otherwise the burst drug release was very high at lower concentrations or polymer precipitation occurs during o/w emulsion preparation at higher concentrations above 12%. Similarly, Labrafil concentrations were kept between 0 and 30% as at higher concentration than 30%, Labrafil precipitation occurs during o/w emulsion preparation.

The initial drug release in ISM is affected by two factors: (i) being administered in liquid form, the drug may diffuse out of the system in parallel with the diffusion of the solvent, prior to polymer solidification (Brodbeck et al., 2000) and (ii) after polymer solidification, the drug associated with the surface or entrapped in polymer matrix will be released rapidly (Luan and Bodmeier, 2006b).

In contrast to previous authors (Luan and Bodmeier, 2006b), higher polymer viscosity accompanied with the use of PLGA 5004 A might be the cause of decrease in burst drug release (0.2 dl/g for PLGA 5002 A vs. 0.4 dl/g for PLGA 5004 A). According to their opinion, a slower solvent diffusion from the low-molecular weight PLGA solution droplets into the release medium lead to a less porous structure of the resulting MPs. In our study, the higher polymer viscosity overrides any other effect and controlled drug burst release. A decrease in the drug release from ISM systems with increasing polymer concentration was already reported due to less porous matrix (Lambert and Peck, 1995). The presence of more oil prolonged the drug diffusion pathway reducing both burst release and release rate.

The use of Labrafil within CZP-loaded MPs and increasing polymer concentration significantly reduces the initial drug release. Several authors elucidated that the high polymer was associated with low burst effect connected with increase the viscosity of organic phase that hamper the drug migration from inner core to outer surface (Fernández-Carballido et al., 2004; Mao et al., 2007; Rahman et al., 2010). Similar to ISM, the burst release is controlled by both polymer and additive concentrations.

Although Labrafil is a PEG derivative, the presence of fatty acids (mainly oleic acid) in the non-ionic amphiphilic compound

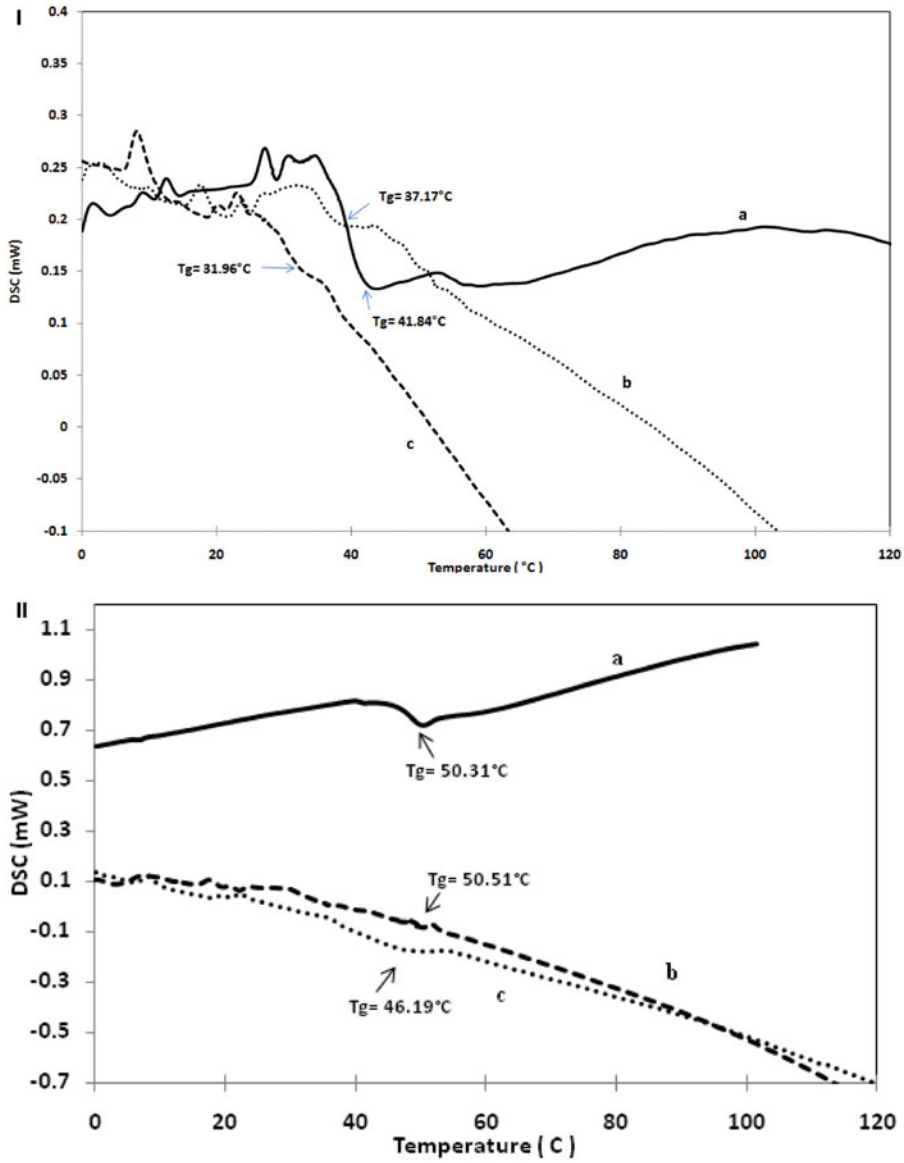

Figure 3. Representative DSC thermographs of the second heating cycle for: I. PLGA 5002 A (a), unloaded PLGA 5002 A ISM (b), CZP-loaded PLGA 5002 A ISM (c) and II. PLGA 5004 A (a), unloaded PLGA 5004 A ISM (b), CZP-loaded PLGA 5004 A ISM (c), showing PLGA glass transition temperature (*Tg*).

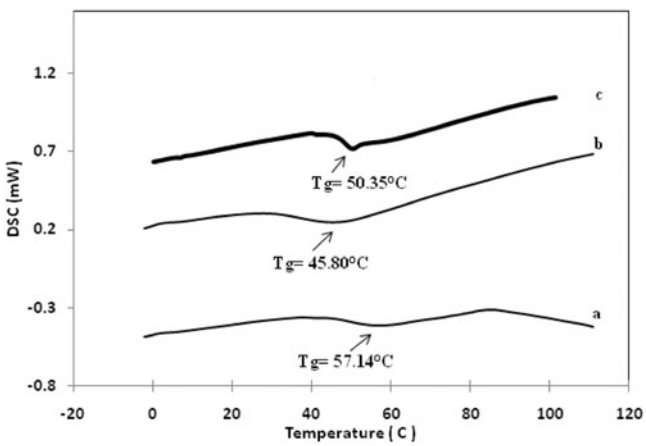

Figure 4. Representative DSC thermographs of the second heating cycle for unloaded PLGA MPs with Labrafil (a), CZP-loaded PLGA MPs with Labrafil (b) and unloaded PLGA MPs without Labrafil (c) showing PLGA glass transition temperature (*Tg*).

granted hydrophobic properties to the molecule. Furthermore, the presence of Labrafil in intimate contact with the polymer in methylene chloride during MPs preparation; promoted channels obstructions, helping in lowering the release rate of the drug (Fernández-Carballido et al., 2004). This actually was proven in our results as Labrafil concentration was the only significant factor responsible for prolonging $T_{80\%}$. Finally, burst release, which is an indication of the amount of drug escaped from MPs and adsorbed on the surface during MP preparation, was affected by both Labrafil and polymer concentration.

In the case of ISM, Korsemeyer–Peppas equation showed unusual small *n* values; an observation generally seen when the equation is used to analyze data of drug release from porous systems (Peppas, 1985). A condition which might be attributed to the rapid drug diffusion, prior to polymer hardening that controlled the drug release after that. The anomalous behaviour of drug release from PLGA MPs indicates a coupled diffusion/polymer relaxation (Colombo et al., 2000).

It was reported that high molecular weight polymers have higher solidification rate than low molecular weight ones (Yang et al., 2001). Accordingly, more porous ISM were formed with PLGA 5002 A than with PLGA 5004 A.

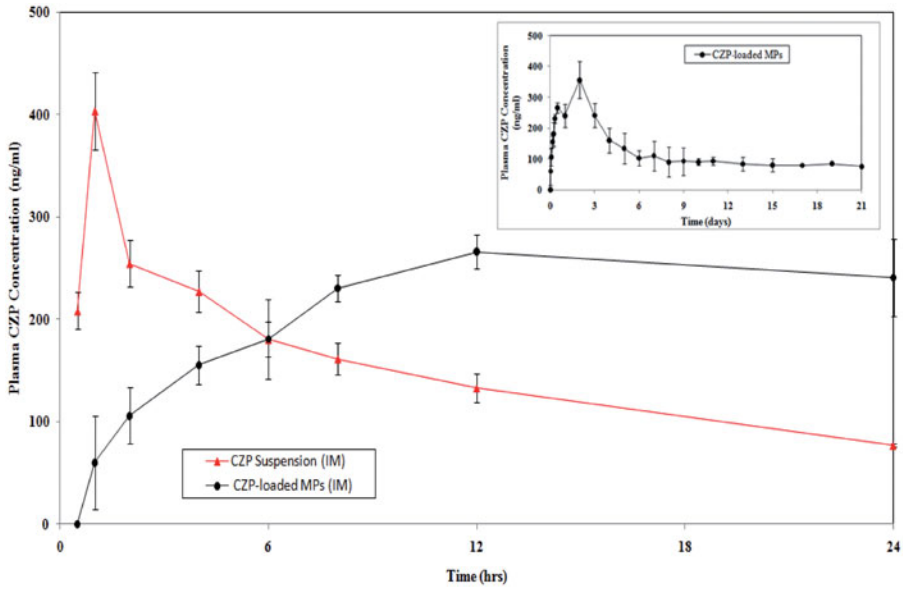

Figure 5. Plasma concentration vs. time curves for CZP after IM administrations of the suspension and MPs (F-9b) at the dose of 20 and 100 mg/kg in rats, respectively. The insert shows the plasma CZP concentration vs. time curves until 21 days after MPs administration. Each point represents mean ± SE ($n = 6$).

Table 5. Pharmacokinetic parameters of CZP after IM administration of suspension and MPs (F-9b).

	Mean value ± SE	
Parameters	CZP Suspension	CZP-loaded MPs (F-9b)
C_{max} (ng/ml)	417.14 ± 83.90	355.84 ± 30.78
T_{max} (h)	1.83 ± 0.83	48.89 ± 2.14
$AUC_{(0–24)}$ (ng h/ml)	3570.85 ± 323.73	–
$AUC_{(0–504)}$ (ng h/ml)	–	61766.80 ± 5247.45
$AUC_{(0–\infty)}$ (ng h/ml)	6247.14 ± 468.34	157638.00 ± 10489.12
$AUMC_{(0–24)}$ (μg h^2/ml)	0.34 ± 0.02	–
$AUMC_{(0–504)}$ (μg h^2/ml)	–	11793.80 ± 652.71
$AUMC_{(0–\infty)}$ (μg h^2/ml)	0.22 ± 0.03	180653.00 ± 15084.15
$t_{1/2}$ (h)	23.55 ± 6.73	871.51 ± 60.24
Clearance (L/h/kg)	3.39 ± 0.29	0.63 ± 0.06
MRT (h)	32.76 ± 9.24	1146.00 ± 96.55

Notes: SE: standard error of the mean, C_{max}: maximum plasma drug concentration, T_{max}: time to maximum drug concentration, AUC: area under the plasma concentration vs. time curve, AUMC: area under the first moment curve, $t_{1/2}$: half-life and MRT: mean residence time.
F-9b: CZP-loaded MPs prepared with 12% PLGA 5004 A and 30% Labrafil.

The shown plasticizing effect of sesame oil on PLGA could be explained by the oil lubrication action between the polymer chains (Wypych, 2004), which is mainly due to the presence of free oleic acid (Kanig and Goodman, 1962; Monedero et al., 2009). The introduction of CZP in ISM had a dual effect on polymer *Tg* depending on the latter molecular weight. The polymer with the lower molecular weight and lower viscosity was subjected to chain relaxation in the presence of the drug, while in the presence of higher molecular weight polymer, the reverse was observed.

Labrafil anti-plasticizing effect on PLGA5004 A containing MPs could be attributed to a decrease in chain polymer mobility caused by a more hydrophobic environment induced by the presence of oil and which was proved in our study by the prolongation of drug release. This may be also the reason for the presence of concavities on the surface of the MPs as shown by SEM (Figure 2b and c). In contrast to ISM, the introduction of the drug showed a plasticizing effect on PLGA 5004 A. So, the method of preparation, the use of different solvents (NMP and DCM) and the type of polymer had an impact on the effect of the drug on chain flexibility.

According to the previous results, it can be concluded that the ISM even with PLGA 5004 A did not achieve a satisfactory control of burst CZP release due to the presence of sesame oil.

MPs prepared with PLGA 5004 A in the presence of Labrafil showed *in vitro* control of burst release with controlled profile and was taken for *in vivo* studies. Sterilization of the selected MPs by gamma irradiation performed in the presence of dry ice prevented polymer degradation that might occur as a result of the increase in temperature taking place during the process and was appropriate for the prepared MPS. Same observations were reported by Herrero-Vanrell et al. (2000); Martinez-Sancho et al. (2004) and Fernández-Carballido et al. (2006).

The Wagner–Nelson method is one of the procedures advised by FDA for the determination of the absorption profile (FDA, 1997). This method is properly used for achieving the absorption profile for rapidly absorbed drugs (AHFS DI, 2010), as there is almost no difference between the amount of drug released and that absorbed. CZP was previously reported to be rapidly absorbed *in vivo* (AHFS DI, 2010).

For controlled-release drug products based only on the polymer degradation, it was reported that the *in vivo* degradation of PLGA polymer is more rapid than that *in vitro* (Machida et al., 2000; Jiang et al., 2003), in addition to the foreign body response (Tracy et al., 1999). While for diffusion–erosion controlled-release systems (anomalous release behaviour of CZP-loaded PLGA MPs), the release of drug depended on both the diffusion of the drug through the polymeric matrix and the polymer degradation. In such case, an appropriate IVIVC could be found. Therefore, the good IVIVC of CZP-loaded PLGA 5004 A MPs was obtained and could be explained by the diffusion–erosion controlled release of CZP from MPs combined with a rapid *in vivo* CZP absorption (AHFS DI, 2010).

Conclusion

The *in vitro* studies revealed that the ESE technique, using Labrafil as a hydrophobic excipient, was more suitable than ISM in controlling CZP burst effect. This was confirmed by the anti-plasticizing effect of Labrafil on PLGA. However, both methods were able to sustain drug release for three weeks with diffusion–erosion mechanism. Furthermore, a high level A IVIVC was

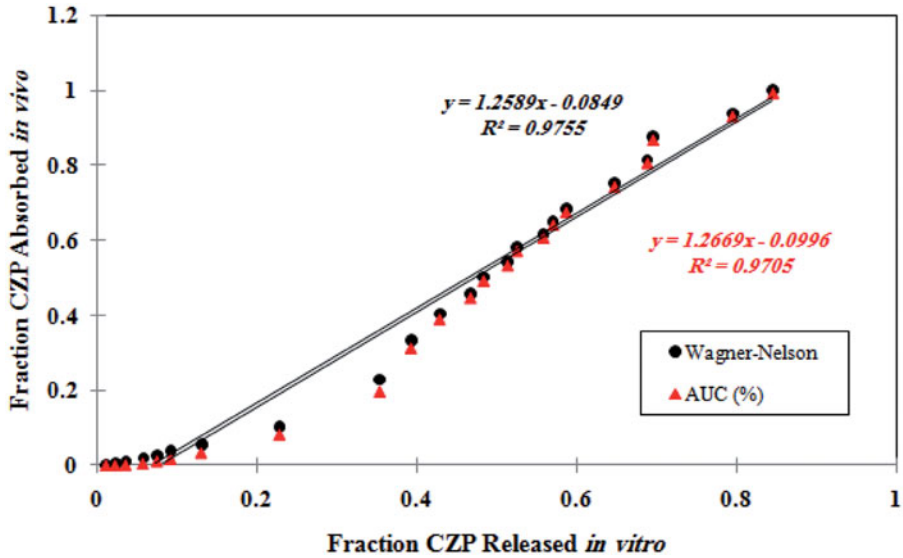

Figure 6. IVIVC model linear regression plots of cumulative absorption and percent AUC vs. percent dissolution of CZP from MPs (F-9b) given IM.

attained ($R^2 = 0.9755$) with the chosen Labrafil-containing MP formula.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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