Pharm Res (2011) 28:1121–1130
DOI 10.1007/s11095-011-0375-z

RESEARCH PAPER

Development and *In Vitro-In Vivo* Evaluation of Polymeric Implants for Continuous Systemic Delivery of Curcumin

Shyam S. Bansal • Manicka V. Vadhanam • Ramesh C. Gupta

Received: 8 October 2010 / Accepted: 19 January 2011 / Published online: 11 February 2011

ABSTRACT

Purpose The introduction of curcumin into clinics is hindered by its low water solubility and poor bioavailability. To overcome these limitations, we developed curcumin implants using poly (ε-caprolactone) as the polymeric matrix.

Methods Implants were prepared by melt-extrusion method; *in vitro* drug release was optimized for effects of polymer composition, drug load, surface area and water-soluble additives. Implants were also tested under *in vivo* conditions for cumulative curcumin release, and liver concentration was correlated with its efficacy to modulate selected xenobiotic-metabolizing enzymes (CYP1A1 and GSTM).

Results Drug release from implants followed biphasic release pattern with Higuchi kinetics and was proportional to the surface area of implants. Drug release increased proportionately from 2 to 10% (w/w) drug load, and incorporation of 10% (w/w) of water-soluble additives (F-68, PEG 8000 and cyclodextrin) did not significantly alter the drug release. *In vivo* drug release was found to be ~1.8 times higher than *in vitro* release. Curcumin was detected at 60 ± 20 ng/g in the liver after four days of implantation and was almost constant (8–15 ng/g) for up to 35 days. This time-dependent drop in curcumin level was found to be due to induction of CYP1A1 and GSTM (μ) enzymes which led to increased metabolism of curcumin.

Conclusion Our data showed that these implants were able to release curcumin for long duration and to modulate liver phase I and phase II enzymes, demonstrating curcumin's biological efficacy delivered *via* this delivery system.

KEY WORDS bioavailability · chemoprevention · controlled release · curcumin · implants

ABBREVIATIONS

ACN	acetonitrile
BCS	bovine calf serum
CYP 1A1	cytochrome P450 1A1
DCM	dichloromethane
DSC	differential scanning calorimetry
ECF	extracellular fluid
FT-IR	Fourier transform infrared spectroscopy
GSTM	glutathione S-transferase (μ)
HPCD	2-hydroxyl propyl β-cyclodextrin
PBS	phosphate-buffered-saline
PCL	poly (ε-caprolactone)
PEG 8K	polyethylene glycol (molecular weight 8,000)
PXRD	powder X-ray diffraction
SEM	scanning electron microscopy

INTRODUCTION

Curcumin, a mixture of three curcuminoids, *viz* curcumin (curcumin I), demethoxycurcumin (curcumin II) and bisdemethoxycurcumin (curcumin III) (Fig. 1), has shown significant potential as a chemopreventive agent in the last

Electronic Supplementary Material The online version of this article (doi:10.1007/s11095-011-0375-z) contains supplementary material, which is available to authorized users.

S. S. Bansal • R. C. Gupta
Department of Pharmacology and Toxicology, University of Louisville
Louisville, Kentucky 40202, USA

S. S. Bansal • M. V. Vadhanam • R. C. Gupta (✉)
James Graham Brown Cancer Center, University of Louisville
Delia Baxter II, Room 304E
580 Preston Street
Louisville, Kentucky 40202, USA
e-mail: rcgupta@louisville.edu

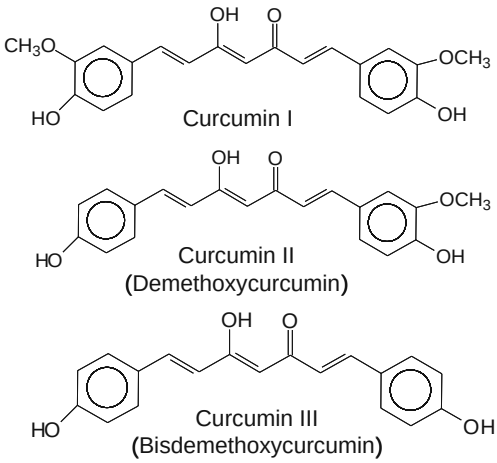

Fig. 1 Structures of the three curcuminoids present in curcumin.

few decades. It is obtained from rhizomes of *Curcuma longa* (turmeric) and is an important ingredient of Indian folklore medicine used in Ayurvedic medicine from 1900 BCE (1). Pubmed shows around 3,000 hits when curcumin is used as a search string, of which ~1,350 are published in the last three years. Curcumin has been found to be a potent inhibitor of NF-kB, AP-1, c-Jun, Jak- STAT pathways and alters the expression of various other cell-signaling pathways in different cancer cell lines (2, 3). This array of activities confers it with potent anti-oxidant, anti-inflammatory, anti-proliferative, anti-metastatic, anti-angiogenic, anti-arthritic, hepatoprotective, anti-diabetic, anti-atherosclerotic, anti-thrombotic, wound healing, and HIV (human immunodeficiency virus) replication inhibition properties and makes it effective in various other ailments of cardiac and pulmonary origin (4). Furthermore, due to inhibition of NF-kB, it has been found to be a potent chemopreventive, able to inhibit carcinogenesis process at its initiation and progression stages induced by environmental carcinogens inducing apoptosis in oncogenic cells (5).

However, despite its high activity in cell culture studies, it has elicited only limited efficacy in various pre-clinical and clinical studies, even at high doses, due to its very poor bioavailability on oral administration. Various studies showed that its low solubility and high rate of metabolism result in limited plasma levels, much below its required therapeutic concentration (6). Although parenteral administration of its high doses in rats helped in achieving therapeutic levels, they were short lived and were found to be cleared within 1–2 h (7). Similar results were also observed in several clinical studies where oral administration of bolus doses (8–12 g/day) given to human volunteers resulted in low plasma concentrations (<1 μg/ml) insufficient to exert any clinically useful pharmacological activity (8–11). To combat this problem and to achieve therapeutic concentrations, various novel drug-delivery systems, like microparticles (12), micelles (13), liposomal vesicles (14), nanoparticles (15), and phospholipid complexes (16), were prepared and tested. But their frequent dosing, invasive administration, inability to achieve high therapeutic concentrations and complex manufacturing requirements limited their introduction into the clinical setting.

Over the past few decades, polymeric implantable drug-delivery systems have shown significant potential for systemic delivery of various therapeutic drugs (17), proteins (18) and enzymes (19) at a controlled rate. These implants are prepared by homogenous entrapment of drugs in a polymeric matrix and can be effectively used to achieve sustained localized delivery with complete bioavailability (20). These polymeric implants were found to be effective for many chemotherapeutic drugs, and several such implants are approved by the FDA for cancer chemotherapy (21). These delivery systems slowly release the encapsulated drug either directly at the tumor site or into the systemic circulation after implantation (22). Furthermore, due to their slow-release kinetics, implants can provide continuous ("24/7") drug release ranging from months to >1 year, which improves the patient compliance (23) while maintaining constant low serum levels of chemopreventives essential for their efficacy.

Therefore, we hypothesized that delivery of curcumin by polymeric implants could enhance its therapeutic efficacy by continuous systemic delivery over an extended period of time. In this paper, we describe design and optimization of curcumin implants prepared using polycaprolactone (PCL) as the polymeric matrix, which has a relatively low melting point and long half-life of degradation (24–26). Drug release was studied and optimized under simulated *in vitro* conditions, and the effect of various parameters was studied in detail. The optimized implants were also tested *in vivo* to determine their rate of release and modulation of selected biomarkers.

MATERIALS AND METHODS

Materials

Dichloromethane, poly (ε-caprolactone) 15,000 and 65,000 molecular weight (PCL-15 and PCL-65), 2-hydroxypropyl β-cyclodextrin and phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich (St. Louis, MO, USA). F-68 was a generous gift from BASF Corporation (Florham Park, NJ, USA). Poly(ethylene glycol) of 8000 molecular weight (PEG-8K) was purchased from Fisher Scientific (Fair Lawn, NJ, USA), ethanol from Pharmco-AAPER (Louisville, KY, USA) and bovine calf serum (BCS) from Hyclone (Logan, UT, USA). Silastic tubing (3.4 mm internal diameter) was purchased from Allied Biomedical (Ventura,

CA, USA), and curcumin (>98% purity) was purchased from Acros Organics (Morris Plains, NJ, USA). All the materials were used as received without any further analysis.

Methods

Preparation of Curcumin Implants

Curcumin implants (2, 5, 10 or 20% w/w) were prepared by melt-extrusion technique. Briefly, the polymer (PCL) and curcumin were dissolved in dichloromethane (DCM) and ethanol, respectively, and mixed together in the presence or absence of a water-soluble polymer to prepare a homogenous dispersion of drug in polymeric matrix. After evaporation of the solvents overnight under vacuum, the molten drug-polymer mixture was extruded using melt extrusion technique through silastic tubing mould attached to a syringe. After a few minutes, the cylindrical implants (3.4 mm diameter) were removed from the tubing, excised into desired lengths (0.5, 1.0, 1.5 and 2.0 cm) and stored at −20°C under argon until use.

Content Uniformity of Implants/Residual Drug Determination

Drug contents in implants ($n=3$) were analyzed to determine the homogeneity of drug distribution and to verify the amount of drug incorporated in each implant. One implant (equivalent to 10 or 20 mg drug/cm implant for 10 or 20% drug load) was weighed and dissolved in 5 ml DCM. Once the implants were dissolved, 5 ml ethanol was added to completely dissolve the drug. The solution was then diluted suitably with ethanol, and drug concentration was measured by UV spectrophotometer at 430 nm.

Residual drug in the implants recovered from animals was also measured similarly, and the average rate of release was calculated as follows:

$$\text{Average daily release} = \frac{\text{Initial amount} - \text{Residual amount}}{\text{Time (days)}}$$

In Vitro Drug Release

Release of curcumin from implants (equivalent to 2, 5, 10 or 20 mg/cm in 2, 5, 10 or 20% w/w) was determined under simulated *in vitro* conditions. Briefly, implants were agitated in 5 ml PBS (pH 7.4) supplemented with 10% (v/v) BCS at 37±0.5°C in a reciprocating shaker water bath (Julabo SW 23, Seelbach, Germany) (150 rpm), and the release medium was changed after every 24 h. PBS was supplemented with BCS (10% v/v) to mimic extracellular fluid composition and to simulate the *in vivo* situation. Albumin present in the BCS binds curcumin via van der Waals forces of interaction and provides perfect sink conditions for release of curcumin (27). Furthermore, $t_{1/2}$ of curcumin degradation was found to increase significantly in the presence of 10% (v/v) BCS (40 h) as compared to PBS (<30 mins) (Supplementary Material Fig. S1). Curcumin concentration was measured spectrophotometrically at 430 nm against a standard curve following the addition of ethanol (10%, final concentration) to ensure complete dissolution of curcumin.

Scanning Electron Microscopy (SEM)

Surface morphology of implants was studied by SEM using Jeol JSM-5310 (Jeol Ltd., Tokyo, Japan). Sections of the implants before and after the *in vitro*/*in vivo* release were cut and mounted on a copper base with carbon glue. The sections were sputter coated with gold using Spi-Module sputter coater (SPI Supplies, West Chester, PA) and analyzed at 25 kV.

In Vivo Drug Release

In vivo drug release was analyzed in female Sprague-Dawley rats following an approved protocol from the Institutional Animal Care and Use Committee (IACUC). After receiving, animals were provided with standard rodent chow diet for seven days and then provided with purified diet AIN-93M until termination of the study. Diets and water were provided *ad libitum*. Two 2-cm implants, containing 10% (w/w) drug load were then subcutaneously grafted at the back of the rats as described (28). Animals were euthanized at different intervals by asphyxiation, and blood, liver and implants were collected. Implants were dried overnight under vacuum and stored at −80°C until analysis of the residual curcumin content. Liver tissue was snap frozen and stored at −80°C for future use.

Analysis of Tissue Curcumin Levels

Liver tissue (~500 mg) from each animal was homogenized in 3 ml PBS (pH 7.4), and 200 µl of 0.5 M sodium acetate was added to reduce the pH to 5. The homogenate was then extracted twice with two volumes of ethyl acetate. After evaporation under vacuum, the residue was reconstituted in 1 ml acetonitrile (ACN). The ACN solution was filtered through 0.45 µ glass-microfiber filter and evaporated under vacuum. The residue was finally reconstituted in 100 µl ACN and analyzed by HPLC using Shimadzu liquid chromatography system equipped with LC-10ADVP pump, RF-10AXL fluorescence detector and a Shimadzu C_{18} column of 5 µm particles (250×4.6 mm). The three curcuminoids were separated by using ACN and 1% citric acid (adjusted to pH 2.5) at a flow rate of 1 ml/min with a gradient elution in which ACN concentration was increased

from 0 to 30% in first 5 min, followed by an increase to 45% in the next 5 to 20 min. ACN was then maintained at this ratio until 36 min. Curcumin detection was achieved using 410 and 500 nm as excitation and emission maxima, respectively, in the fluorescence detector.

Microsome Extraction

Liver (~500 mg) was homogenized in 0.25 M sucrose buffer (pH 7.4) at 3,000 rpm with Polytron. The homogenate was centrifuged at 3,000 g for 20 min at 4°C to separate the nuclear content. The supernatant was collected and again centrifuged at 11,000 g for 20 min at 4°C to separate the mitochondria. The supernatant was then transferred to ultracentrifuge tubes and finally centrifuged at 100,000 g for 1 h at 4°C to separate the microsomes. The pellet was suspended in 1 ml sucrose buffer, aliquoted and stored at −80°C until use.

Analysis of Cytochrome P450s and GSTμ (GSTM)

Microsomal proteins were quantified using bicinchoninic acid (BCA) method (29) using BCA™ Protein Assay kit (Thermo Scientific, Rockford, IL, USA) and were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel. Protein bands were then transferred to polyvinylidene diflouride (PVDF) membrane, which was incubated with 5% non-fat dried milk in Tris-buffered saline for 1 h at room temperature (25°C) to block the non-specific binding sites. The membrane was then incubated with primary antibodies for CYP1A1 and GSTM, followed by horseradish peroxidase conjugated secondary antibody. The bands were detected by enhanced chemiluminescence using Pierce® ECL Western-Blotting Substrate (Thermo Scientific, Rockford, IL, USA) and quantified using VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Coomasie blue stained gel was used as a loading control and is given as Supplementary Material Fig. S2.

RESULTS AND DISCUSSION

Due to the low melting point of PCL (60°C), curcumin implants were prepared by melt-extrusion technique at 65°C. No residual solvents were found by gas chromatography (data not shown). Curcumin was found to be stable as determined by HPLC, and polymer was found to be stable by differential scanning calorimetry (Supplementary Material Fig. S2). Drug-polymer blend showed only one melting endotherm at 58°C corresponding to PCL polymer, and no melting endotherm corresponding to curcumin was observed at 10% drug load (10 mg/cm). However, slight crystallization was observed at 20% w/w drug load. Similarly, a halo pattern with only two peaks characteristic of PCL (due to its semi-crystalline nature) was observed in 10% drug load implants by powder X-ray diffraction studies (PXRD) (Supplementary Material Fig. S3). The absence of curcumin melting endotherm by DSC and appearance of a halo pattern by PXRD in the implants containing 10% curcumin revealed that the drug was dispersed at a molecular level in the polymer matrix and was stabilized in its amorphous form by the polymer (30). Furthermore, Fourier transform infrared spectroscopy (FT-IR) studies showed that a small fraction of curcumin molecules existed as keto-enol tautomers. In the presence of PCL, these keto-enolic -OH groups of curcumin interact with –C = O group of PCL via formation of H bonds (data not shown).

Drug Content Analysis

To ensure the homogenous distribution and batch-to-batch reproducibility of curcumin in the polymer matrix, 1-cm implants (10 mg drug/cm implant) were dissolved in DCM: ethanol (1:1), and curcumin content was measured in triplicate. Analysis of drug content in implants showed that the implants were uniform with homogenous dispersion of drug. Almost 95%±1.2% drug was recovered from PCL implants prepared in the same batch or different batches, with negligible variability in drug loading between the implants prepared at different times. This showed that curcumin implants prepared under these conditions were reproducible with respect to drug content and drug distribution.

In Vitro Drug Release: Effect of Polymer Molecular Weight

Since molecular weight of polymers is one of the determining factors for rate of influx and efflux of extracellular fluid through the polymeric matrix (31), different implants were prepared by blending PCL of 15,000 (PCL-15) and 65,000 (PCL-65) molecular weights in different proportions with 10% drug load (10 mg/cm). The implants were then analyzed for their daily drug release for up to ~140 days (Fig. 2). Data showed that initially ~17% drug was released from PCL-15 implants in one week, as compared to around 13% from PCL-65 implants, and decreased slightly with increasing concentration of PCL-65. Daily drug release was slightly higher from PCL-15 implants during the first week, as compared to PCL-65, after which it was similar from both the implants up to 60 days. However, after ~60 days, drug release was higher from PCL-65 implants than PCL-15. Daily release from PCL-65 implants was ~3 times higher (30–35 μg/day) than PCL-15 implants (10–12 μg/day) after 100 days. It appears that the pore size of the

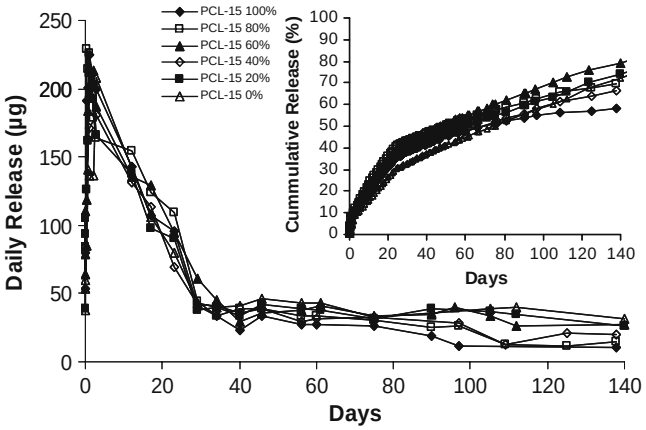

Fig. 2 Effect of polymer composition on drug release. Data denote average of three replicates. SD, generally 5–10%, has been excluded for clarity. Implants were prepared by blending different proportions of PCL-15 (poly(ε caprolactone)) with 15000 molecular weight and PCL of 65000 molecular weight, and release rate was determined.

polymeric matrix of both PCL-15 and PCL-65 is greater than the molecular size of the curcumin molecules and, hence, is not the limiting factor for the drug diffusion during initial period. However, with time, when the path length of solvent diffusion increases, PCL-65 offers least resistance to the drug release; hence, the drop in daily drug release is minimal with PCL-65 implants (32).

This study also showed that drug release from these polymeric implants follow biphasic release kinetics. A burst release was observed initially, which declined rapidly during the first 25 days followed by a much slower decline for next 100 days. Two hundred to 250 µg curcumin was found to be released on day 1, which then declined with time and dropped below 100 µg/day after 20 days. This burst release could be due to release of surface-bound drug, followed by a more controlled release from the inner layers of implant's polymer matrix. Such a burst effect can be useful in cases where rapid achievement of plasma concentration of therapeutic agents is desired and which can then be maintained with slow controlled release of drugs (33). However, in cases where such a burst release is undesirable, these implants can be incubated in ethanol for 1–2 h to get rid of most of the surface-bound drug (data not shown). Furthermore, the decline in drug release during the diffusion from the inner layers of polymeric matrix could be attributed to either reduction in drug's permeability due to increased crystallinity of polymer and/or decrease in concentration gradient of drug in outer layers of polymer matrix (31). It is known that due to the semi-crystalline nature of PCL, its crystallinity increases with time in the presence of aqueous release media, which might result in increased resistance for diffusion of aqueous fluids and, hence, decreasing the drug release with time (34). Moreover, a decrease in concentration gradient of drug in outer polymer layers results in longer path length for diffusion of aqueous fluid into deeper layers, which also results in a decrease in drug release (34). However, when cumulative release data (Fig. 2 inset) from these implants was analyzed by zero-order, first-order, Higuchi and Hixson Crowell models for drug-release kinetics (35), it was found that drug release from PCL implants followed diffusion-mediated Higuchi kinetics ($r^2 > 0.98$). It shows that PCL implants are able to release drug at a controlled rate over an extended period of time, and the drop in drug release was indeed partly due to longer path length of diffusion (35).

The implants were also analyzed by SEM before and after the *in vitro* release. The freshly prepared implants were found to be smooth with homogenous dispersion of drug without any visible evidence of discontinuities. No significant difference was observed in the polymeric matrix 140 days after the *in vitro* release, which showed that the polymeric matrix was stable in the release medium for at least this period of time. However, a small amount of drug crystallization was observed in these implants, which might be due to slow crystallization of amorphous drug in the polymer matrix that can occur when it comes in contact with the aqueous release media (Fig. 3a-c).

Effect of Drug Loading

These implants were also analyzed for the effect of drug loading on daily drug release. It has been found that due to channeling effect, increasing drug loads lead to higher drug release (36). Therefore, implants with 2%, 5%, 10%, 20%, 30%, 40% and 50% drug load (2, 5, 10, 20, 30, 40 and 50 mg per cm implant, respectively) were prepared and analyzed for daily drug release. Data from 2-20% drug load implants is shown in (Fig. 4). It was found that drug release was proportional to drug concentration for only up to 10% drug load, and no further increase in the release was observed with higher drug loads. Daily drug release was ~2–3 times higher from 5% implants as compared to 2% implants and similarly was 1.7–1.9 times higher from 10% drug load implants as compared to 5% drug load at all time points. It appears that only up to 10% drug load, the channeling effect increases, enhancing the porosity of matrix for diffusion of release media. But, at higher drug loads, solubility of curcumin in the polymeric matrix becomes a limiting factor. Furthermore, due to lipophilic nature of curcumin, increasing drug concentration leads to an increase in lipophilicity of the curcumin-polymer matrix, which could also be a contributing factor leading to retardation of influx of release medium (32). SEM analysis of these implants showed that at drug loads >10%, degree of discontinuities and extent of drug crystallization in the polymeric matrix also increased proportionately (Fig. 3d, e and f), which could also be a limiting factor.

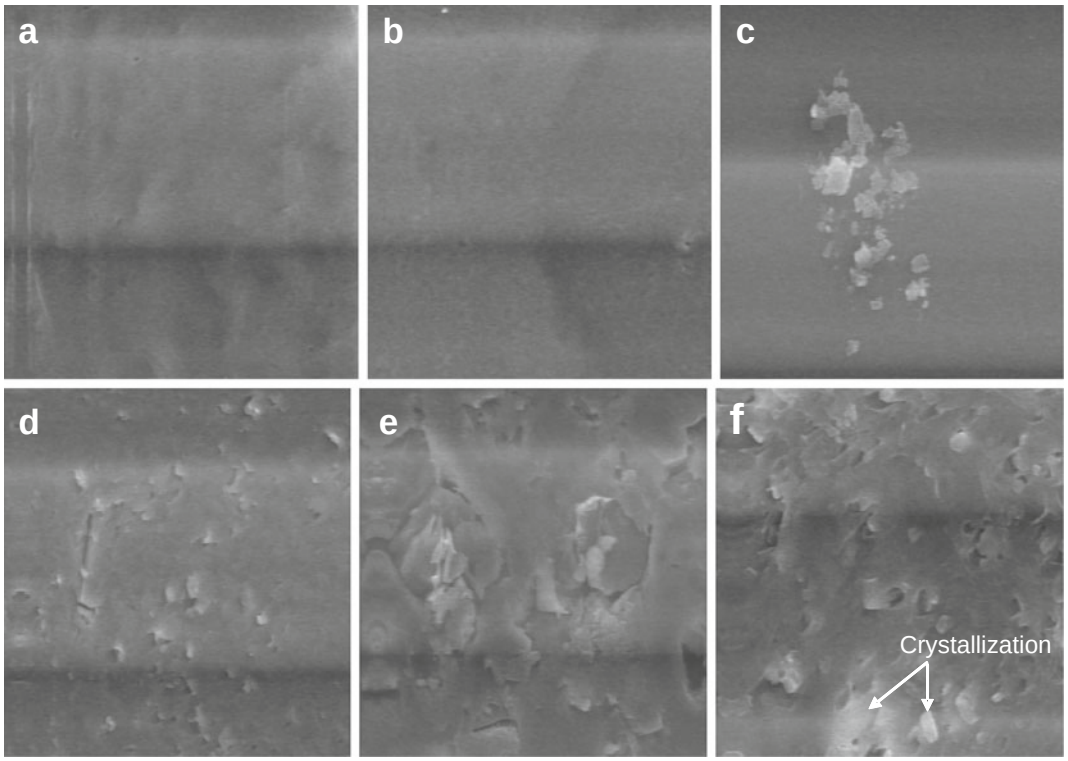

Fig. 3 Scanning electron microscopy (SEM) photographs (1,000X magnification) of blank implant (polymer alone) (**a**), implant with 10% drug load (**b**), implant with 10% drug load after 140 days of *in vitro* release (**c**), implants with 20% drug load (**d**), implants with 30% drug load (**e**), and implants with 20% drug load showing traces of crystallization (**f**) (2,000X magnification).

Effect of Formulation Additives

Addition of certain excipients in the polymeric matrix influences drug release significantly. They may increase or decrease the drug release rates by changing the matrix lipophilicity, matrix porosity, microclimate pH and or polymer drug interactions (37, 38). Excipients, especially hydrophilic polymers compatible with the polymeric matrix, are helpful in providing aqueous channels to facilitate the diffusion-mediated drug release. Due to their water solubility, such hydrophilic excipients preferentially dissolve in the ECF and form a porous matrix, enhancing the drug release (39). This effect is more profound in case of less permeable polymeric matrices, especially where drug release is dictated by diffusion through the polymer. Furthermore, a reduction in implant's rigidity and viscosity due to a relative decrease in matrix-forming polymer content (due to addition of water-soluble polymers) might also be a contributing factor towards enhancement of drug release.

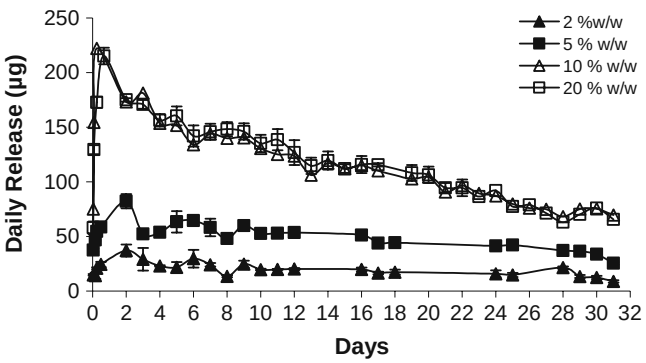

Fig. 4 Effect of drug loading (2%, 5%, 10% and 20%, w/w) on daily drug release. Each 1-cm implant contained 2, 5, 10 or 20 mg drug, respectively. Data presented denote average of three replicates (±SD).

Therefore, we studied the effect of several water-soluble polymeric additives for their influence on drug release. Implants were prepared using PCL-65, 20% drug load and 10% of either F-68, PEG-8,000 or 2-hydroxypropyl β-cyclodextrin (HPCD), and the drug release was compared with the control implants prepared without any such additives (Fig. 5). It was observed that the presence of 10% F-68 or PEG decreased the drug release to some extent. Implants prepared without any additive or with HPCD showed 12% drug release over a period of 31 days as compared to 9.5% drug release from implants prepared with F-68 or 11% with PEG. Since no significant difference in drug release was observed with 10% water-soluble additives and the presence of F-68 was found to facilitate the extrusion by significantly reducing the viscosity of polymeric mixtures, it was retained as a formulation additive in all subsequent studies.

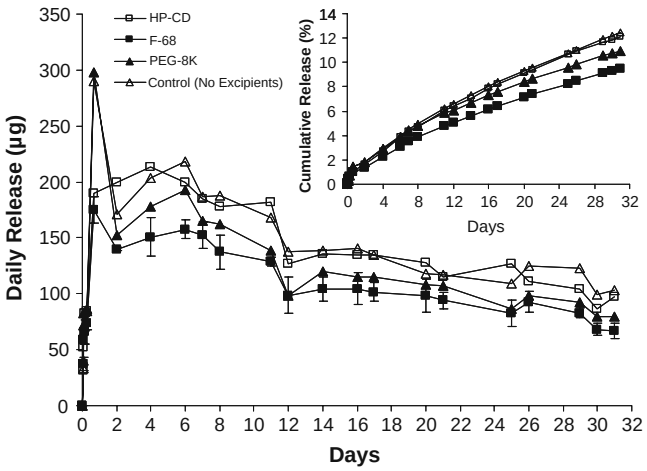

Fig. 5 Effect of water-soluble additives on daily drug release. Data presented denote average of three replicates of 1-cm implants containing 20 mg drug. SD, generally 5–10%, has been excluded for clarity and is shown only for one set of implants.

Effect of Surface Area

Since the drug release from implants was found to follow Higuchi kinetics, it was expected that an increase in surface area should proportionately increase the drug release. Therefore, to study the effect of surface area, implants of different sizes were prepared—0.5 cm with a surface area of 0.69 cm^2 (10 mg drug), 1 cm with a surface area of 1.21 cm^2 (20 mg drug) and 2 cm with a surface area of 2.24 cm^2 (40 mg drug)—and analyzed for daily drug release (Fig. 6). As expected, a proportionate increase in drug release was observed with increasing surface area. Daily drug release from 1-cm implants was ~1.6 to 1.8 times higher as compared to 0.5-cm implants after one week. Simlarly, daily drug release from 2-cm implants was 1.3 to 1.5 times higher as compared to 1-cm implants. Since drug release from these implants was diffusion mediated, according to Fick's law of diffusion, as the size of the implants increases, the effective surface area for diffusion of extracellular fluid increases, which, in turn, increases the amount of drug efflux from the polymeric matrix (40). Therefore, in order to have maximum drug release, 2-cm implants were selected for further studies.

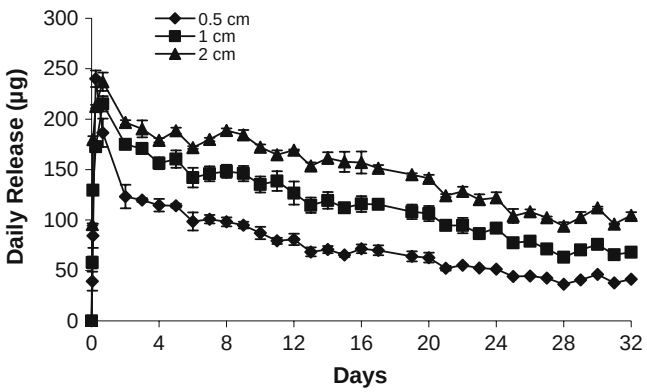

Fig. 6 Effect of surface area on daily drug release. Data represent average of 3 implants (±SD) with 20 mg drug/cm.

In Vivo Drug Release

Drug release from implants is a dynamic process that largely depends upon the surrounding environment and polymer characteristics. Extracellular fluid composition and volume can drastically alter the rate and extent of drug release. Furthermore, the presence of other components, such as collagen, plasma proteins, and lipids, determines the drug distribution in the tissues and drug partitioning into the plasma (33). All these factors warrant drug release studies to be done under *in vivo* conditions and to determine at what rate and extent these polymeric implants can release the drug and maintain their integrity under more stringent *in vivo* conditions. Therefore, 2-cm implants (10% w/w drug load) were grafted subcutaneously beneath the skin of female S/D rats and were recovered following euthanasia after predetermined time intervals for a period of five weeks to determine the residual drug content (Fig. 7) and to derive an *in vitro-in vivo* correlation.

As evident from Fig. 7, these implants exhibited similar behavior under *in vivo* conditions as was observed under *in vitro* conditions. A burst release of ~2.64 mg was observed in the first week, corresponding to an average daily release of 377 µg. However, release dropped to 313 µg per day in the next week and continued decreasing with time, although at a slower rate. This *in vivo* release trend is in concordance with the *in vitro* release rates, except that the average *in vivo* daily release was found to be nearly 1.8-fold higher than *in vitro* release in 5 ml media. Furthermore, comparison of *in vivo* drug release from 10% or 20% drug load revealed that the burst effect was almost double with 20% drug load as compared to 10% drug load. The subsequent release

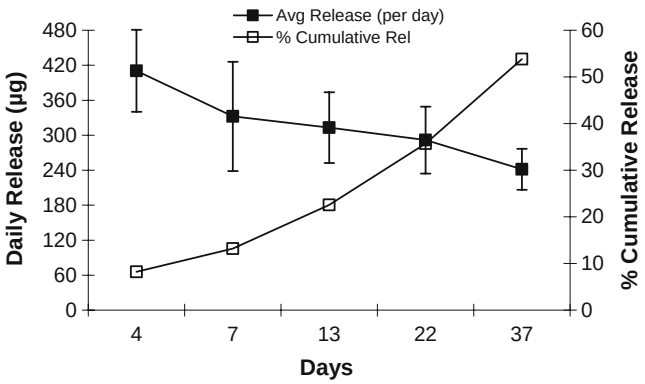

Fig. 7 *In vivo* release of curcumin from each 2-cm implant with 10% (w/w) (equivalent to 10 mg drug/cm implant) curcumin load ($n = 3$). Implants were grafted subcutaneously in Sprague-Dawley rats and were recovered at indicated time intervals to measure the residual curcumin content.

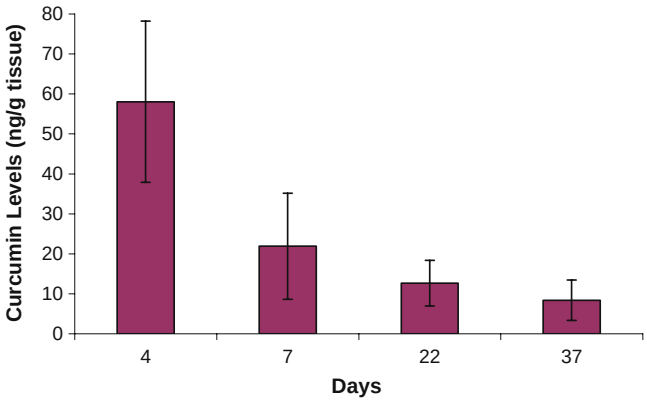

Fig. 8 Curcumin distribution in liver tissue of Sprague-Dawley rats administered by two 2-cm polymeric implants each containing 20 mg drug. Curcumin was extracted with ethyl acetate from tissues and analyzed by HPLC equipped with a fluorescence detector.

kinetics following the burst effect were essentially similar with the two drug loads, as was observed under *in vitro* conditions. Moreover, intact nature of these implants after 16 months under *in vivo* conditions showed that these implants were physically stable under these conditions and can efficiently deliver curcumin for at least 1.5–2 years.

Analysis of Drug Distribution in Tissues

The pharmacokinetics of drug disposition often depend upon the route of administration. It is well known that when curcumin is administered orally, it exhibits poor bioavailability due to poor absorption coupled with high first-pass metabolism (2, 7). Moreover, curcumin, being a lipophilic compound, is known to possess high volume of distribution (41). Therefore, in order to ensure the efficacy of these polymeric implants, curcumin was extracted and analyzed in liver tissue (a highly perfused organ), instead of plasma, of rats implanted with two 2-cm implants with 10% drug load (10 mg/cm). It was found that ~60±20 ng/g (0.16 μM considering liver density equal to water) of curcumin was present in the liver after 4 days of implantation, which dropped with time and remained almost constant ~8–15 ng/g (0.025 to 0.04 μM) from 7 days to 37 days (Fig. 8).

These results showed that polymeric PCL implants were able to efficiently release the drug which gets distributed to distant organs through the systemic circulation. Although curcumin levels were not measured in the local tissue, visual inspection of implant site showed significantly higher levels of (yellow) pigmented curcumin, which can act as a tissue-storage site ("tissue depot") from which constant amounts of drug can diffuse into the systemic circulation, irrespective of the rate of release from the implants (not shown).

Curcumin is known to be a potent inducer of phase I and phase II enzymes, including CYP1A1 and glutathione-S-transferase mu (GSTM), which are also responsible for its metabolism (42, 43). It appears that induction of these enzymes with initial high doses of curcumin leads to an enhanced curcumin metabolism which decreases the liver concentration with time. However, with time, when there is no further induction, almost constant levels of curcumin were observed. In order to further substantiate these results, we analyzed CYP1A1 and GSTM in the liver tissue by Western-blot analysis. Since curcumin concentration was highest at 4 and 7 days after implantation with 10% drug load implants, microsomes were isolated from liver tissues from these time points. The microsomal proteins were then separated on polyacrylamide gels, and CYP1A1 and GSTM levels were quantified (Fig. 9).

Analysis of CYP1A1 and GSTM levels as the biomarkers of curcumin efficacy showed an induction of 13- and 3-fold in CYP1A1 and GSTM levels, respectively, after 4 days of implantation. After 7 days, the levels somewhat increased to 16- and 4-fold, respectively. These data showed that decreased levels of curcumin in liver with time were indeed due to an increase in its metabolism due to sustained induction of phase I and phase II enzymes. It, therefore, would be interesting to determine the effect of incorporating piperine-like compounds into the curcumin implants that can inhibit curcumin metabolism and hence could further enhance curcumin levels in tissue, as was shown by Shoba *et al.* (44).

Overall, the data presented demonstrate that considerably higher amounts of curcumin can be delivered to distant organs from subcutaneous implants compared to the traditional oral route and could provide a viable alternative for the delivery of such potent chemopreventives in target organs where surgical implantation of such

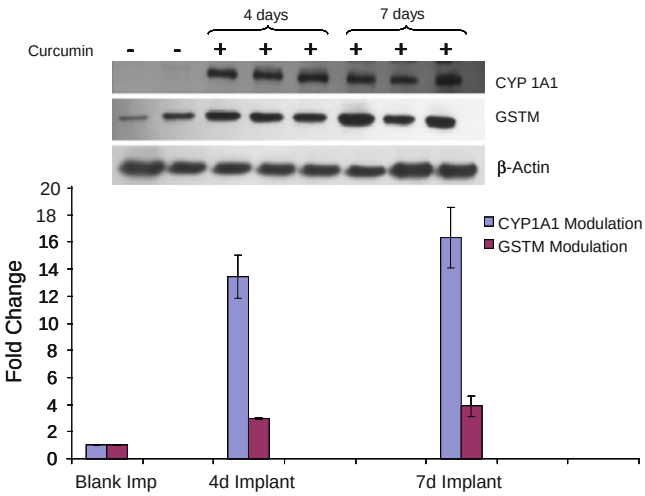

Fig. 9 Western-blot analysis of liver tissue for CYP1A1 and GSTM levels 4 and 7 days after subcutaneous implantation of two 2-cm curcumin implants each containing 20 mg drug. β-Actin was used as loading control, and Coomassie blue stained gel was used to visualize modulation of various proteins (supplementary Fig. 4).

delivery devices in desired organs (brain, liver, breast tissue) is feasible.

CONCLUSIONS

In this study, we prepared and optimized polymeric implants of curcumin to bypass the oral route and to enhance its efficacy by continuously delivering curcumin at a controlled rate, directly into the systemic circulation. Curcumin, due to its lipophilic nature, was found to be miscible with the polymer and showed a diffusion-mediated biphasic release pattern in accordance with Higuchi kinetics. Drug release was found to increase with increasing drug load for up to 10% (w/w) and was constant at higher drug loads (>10%, w/w). Analysis of liver tissue showed presence of ~60 ng/g of curcumin 4 days after implantation, which then seemed to plateau at 8–15 ng/g after 7 days. Furthermore, presence of a tissue depot of curcumin at the site of implantation implies an almost constant influx of drug into the systemic circulation even after prolonged periods of time. Sustained higher levels of CYP1A1 and GSTM proteins in liver microsomes are reflective of constant exposure to curcumin. This study showed that polymeric implants could be a viable alternative for the delivery of potent chemopreventives like curcumin and afford a continuous ("24/7") delivery system. Furthermore, this approach could also be useful in analyzing the chemosensitivity potential of curcumin or its efficacy in metronomic chemotherapy with and without other chemotherapeutics.

ACKNOWLEDGMENTS

This research work was supported from USPHS grants CA-118114, CA-125152, CA-90892, and from Agnes Brown Duggan Endowment. R.C.G. is Agnes Brown Duggan Chair in Oncological Research. Dr. Hina Kausar is acknowledged for her assistance in the western blot studies.

REFERENCES

1. Bar-Sela G, Epelbaum R, Schaffer M. Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. Curr Med Chem. 2010;17:190–7.
2. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 2003;23:363–98.
3. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, *et al.* Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol. 2008;76:1590–611.
4. Aggarwaland BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol. 2009;41:40–59.
5. Aggarwal BB. Apoptosis and nuclear factor-kappa B: a tale of association and dissociation. Biochem Pharmacol. 2000;60:1033–9.
6. Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug Metab Dispos. 1999;27:486–94.
7. Holder GM, Plummer JL, Ryan AJ. The metabolism and excretion of curcumin (1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione) in the rat. Xenobiotica. 1978;8:761–8.
8. Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, *et al.* Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol. 2008;28:110–3.
9. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, *et al.* Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 2001;21:2895–900.
10. Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL, *et al.* Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res. 2008;14:4491–9.
11. Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, *et al.* Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. Br J Cancer. 2004;90:1011–5.
12. Shahani K, Swaminathan SK, Freeman D, Blum A, Ma L, Panyam J. Injectable sustained release microparticles of curcumin: a new concept for cancer chemoprevention. Cancer Res. 2010;70:4443–52.
13. Ma Z, Haddadi A, Molavi O, Lavasanifar A, Lai R, Samuel J. Micelles of poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization, stabilization, and controlled delivery of curcumin. J Biomed Mater Res A. 2008;86:300–10.
14. Thangapazham RL, Puri A, Tele S, Blumenthal R, Maheshwari RK. Evaluation of a nanotechnology-based carrier for delivery of curcumin in prostate cancer cells. Int J Oncol. 2008;32:1119–23.
15. Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MN. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci. 2009;37:223–30.
16. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm. 2007;330:155–63.
17. Jain JP, Modi S, Domb AJ, Kumar N. Role of polyanhydrides as localized drug carriers. J Control Release. 2005;103:541–63.
18. Hafeman AE, Li B, Yoshii T, Zienkiewicz K, Davidson JM, Guelcher SA. Injectable biodegradable polyurethane scaffolds with release of platelet-derived growth factor for tissue repair and regeneration. Pharm Res. 2008;25:2387–99.
19. Ghalanbor Z, Korber M, Bodmeier R. Improved lysozyme stability and release properties of poly(lactide-co-glycolide) implants prepared by hot-melt extrusion. Pharm Res. 2010;27:371–9.
20. Kumar Naraharisetti P, Yung Sheng Ong B, Wei Xie J, Kam Yiu Lee T, Wang CH, Sahinidis NV. *In vivo* performance of implantable biodegradable preparations delivering Paclitaxel and Etanidazole for the treatment of glioma. Biomaterials. 2007;28:886–94.
21. Saltzmanand WM, Fung LK. Polymeric implants for cancer chemotherapy. Adv Drug Deliv Rev. 1997;26:209–30.
22. Dhanikulaand AB, Panchagnula R. Localized paclitaxel delivery. Int J Pharm. 1999;183:85–100.

23. Dashand AK, Cudworth 2nd GC. Therapeutic applications of implantable drug delivery systems. J Pharmacol Toxicol Methods. 1998;40:1–12.
24. Li C, Cheng L, Zhang Y, Guo S, Wu W. Effects of implant diameter, drug loading and end-capping on praziquantel release from PCL implants. Int J Pharm. 2010;386:23–9.
25. Pitt CG, Jeffcoat AR, Zweidinger RA, Schindler A. Sustained drug delivery systems. I. The permeability of poly(epsilon-caprolactone), poly(DL-lactic acid), and their copolymers. J Biomed Mater Res. 1979;13:497–507.
26. Woodruffand MA, Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. Progress in Polymer Science. In Press, Corrected Proof.
27. Quitschke WW. Differential solubility of curcuminoids in serum and albumin solutions: implications for analytical and therapeutic applications. BMC Biotechnol. 2008;8:84.
28. Ravoori S, Vadhanam MV, Sahoo S, Srinivasan C, Gupta RC. Mammary tumor induction in ACI rats exposed to low levels of 17beta-estradiol. Int J Oncol. 2007;31:113–20.
29. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, *et al.* Measurement of protein using bicinchoninic acid. Anal Biochem. 1985;150:76–85.
30. Bansal SS, Kaushal AM, Bansal AK. Co-relationship of physical stability of amorphous dispersions with enthalpy relaxation. Pharmazie. 2008;63:812–4.
31. Lemmouchi Y, Schacht E, Lootens C. *In vitro* release of trypanocidal drugs from biodegradable implants based on poly ([var epsilon]-caprolactone) and poly(-lactide). J Control Release. 1998;55:79–85.
32. Rothen-Weinhold A, Besseghir K, Gurny R. Analysis of the influence of polymer characteristics and core loading on the *in vivo* release of a somatostatin analogue. Eur J Pharm Sci. 1997;5:303–13.
33. Weinberg BD, Blanco E, Gao J. Polymer implants for intratumoral drug delivery and cancer therapy. J Pharm Sci. 2008;97:1681–702.
34. Pitt CG, Gratzl MM, Jeffcoat AR, Zweidinger R, Schindler A. Sustained drug delivery systems II: factors affecting release rates from poly(epsilon-caprolactone) and related biodegradable polyesters. J Pharm Sci. 1979;68:1534–8.
35. Costaand P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13:123–33.
36. Rosenberg Rachel T, Siegel Steven P, Dan N. Effect of drug loading on the rate of nicotine release from poly(?-caprolactone) matrices. Polymer degradation and performance, vol. 1004. American Chemical Society; 2009. pp. 52–59.
37. Desai KG, Mallery SR, Schwendeman SP. Effect of formulation parameters on 2-methoxyestradiol release from injectable cylindrical poly(DL-lactide-co-glycolide) implants. Eur J Pharm Biopharm. 2008;70:187–98.
38. Kipp J, Rabinow B, Gokarn Y. Excipient selection and criteria for injectable dosage forms. Excipient development for pharmaceutical, biotechnology, and drug delivery systems, vol. null. Informa Healthcare, 2006.
39. Ma G, Song C, Sun H, Yang J, Leng X. A biodegradable levonorgestrel-releasing implant made of PCL/F68 compound as tested in rats and dogs. Contraception. 2006;74:141–7.
40. Zhang X, McAuley KB, Goosen MFA. Towards prediction of release profiles of antibiotics from coated poly(-lactide) cylinders. J Control Release. 1995;34:175–9.
41. Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol. 2007;595:453–70.
42. Goud VK, Polasa K, Krishnaswamy K. Effect of turmeric on xenobiotic metabolising enzymes. Plant Foods Hum Nutr. 1993;44:87–92.
43. Safe S, Wang F, Porter W, Duan R, McDougal A. Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. Toxicol Lett. 1998;102–103:343–7.
44. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 1998;64:353–6.