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Research Article

Biodegradable Injectable *In Situ* Implants and Microparticles for Sustained Release of Montelukast: *In Vitro* Release, Pharmacokinetics, and Stability

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Abstract. The objective of this study was to investigate the sustained release of a hydrophilic drug, montelukast (MK), from two biodegradable polymeric drug delivery systems, *in situ* implant (ISI) and *in situ* microparticles (ISM). *N*-Methyl pyrrolidone (NMP), dimethyl sulfoxide (DMSO), triacetin, and ethyl acetate were selected as solvents. The release of 10% (*w*/*v*) MK from both systems containing poly-lactic-co-glycolic acid (PLGA) as the biodegradable polymer was compared. Upon contact with the aqueous medium, the PLGA in ISI and ISM systems solidified resulting in implants and microparticles, respectively. The *in vitro* drug release from the ISI system showed marked difference from miscible solvents (NMP and DMSO) than the partially miscible ones (triacetin and ethyl acetate), and the drug release decreased with increased PLGA concentration. In the ISM system, the initial *in vitro* drug release decreased with decreased ratio of polymer phase to external oil phase. *In vivo* studies in rats showed that ISM had slower drug release than the drug release from ISI. Also, the ISM system when compared to ISI system had significantly reduced initial burst effect. *In vitro* as well as the *in vivo* studies for both ISI and ISM systems showed sustained release of MK. The ISM system is suitable for sustained release of MK over 4-week period with a lower initial burst compared to the ISI system. Stability studies of the ISI and ISM formulations showed that MK is stable in the formulations stored at 4°C for more than 2 years.

KEY WORDS: controlled release; *in vitro* studies; *in vivo* studies; leukotriene receptor antagonist; polymeric drug delivery.

INTRODUCTION

The current trend toward developing sustained release injectable formulations such as microspheres, solid implants, or gel systems has been increased due to several advantages of these systems such as site-specific action, reduced side effects, and improved patient compliance (1). Some of the limitations of microspheres are low drug loadings and difficulty in particle size control, while the solid implants may require surgery for insertion or removal from body. *In situ* implants (ISI) or *in situ* microparticles (ISM) systems have been introduced to overcome these limitations (2) in addition to their various biomedical applications (3–5).

In the ISI system, a biodegradable polymer is dissolved in a biocompatible solvent. The drug may be dissolved or suspended in the polymer solution (polymer phase). Solvents such as 2-*N*-methyl pyrrolidone (NMP), dimethyl sulfoxide (DMSO), and 2-pyrrolidone can be used to get highly concentrated polymer solution (6). After injection in the body, the polymer forms *in situ* implants and sustains the release of the entrapped drug. Several mechanisms such as solvent exchange, pH change, UV irradiation, ionic cross-linking, temperature transition, and chemical reactions may lead to the *in situ* implant formation (7–9). The type of polymer used plays an important role in the formulation of these long acting drug delivery systems and can significantly affect the release rate of drugs. Among those, biodegradable polymers are preferred as surgical removal of the implant is not required. Some of the biodegradable polymers that may be used for *in situ* implants are carbopol 934, HPMC, poly-lactic acid, poly-lactic-co-glycolic acid (PLGA), poly-e-caprolactone, alginic acid, chitosan derivatives, poly-vinyl alcohol, poly-vinyl derivatives, and pectin.

ISM system has been developed as sustained delivery of drugs and to overcome the limitations of pre-formed microspheres or microparticles (10,6). In the ISM system, a polymeric solution containing the drug (polymer phase) is emulsified into an outer oil phase such as peanut oil. After administration inside the body, the internal polymer phase solidifies to form microparticles, and the drug release from the microparticles is sustained (10,6).

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Montelukast sodium (MK) is a selective leukotriene receptor antagonist. The drug is useful in the management of chronic asthma, prophylaxis of exercise-induced asthma, and treatment of other conditions where leukotriene is implicated such as capsular contracture and obstructive sleep apnea (11–13). Montelukast has a small daily dose of 10 mg/day in adults and 4–5 mg/day in children. It should not be used to treat an acute asthma attack (13). The bioavailability of MK after oral administration is around 62%, and the half-life of MK is 6.7 h in the human (14).

MK is not available in the market as a parenteral long acting pharmaceutical formulation. A sustained release formulation may provide better efficacy and compliance in the therapy of chronic asthma, exercise-induced asthma conditions, and other treatments. The ISI and ISM formulations provide sustained release of drugs and can be administered by both intramuscularly and subcutaneously. These systems are much easier to prepare and administer than surgical implants and typical microparticles.

The objective of this study was to develop ISI and ISM formulations for 1-month sustained release of MK. Parameters such as solvent type and appropriate concentration of polymer for both ISI and ISM systems were evaluated for the sustained release of MK. Selected formulations from *in vitro* release studies were used to study the pharmacokinetics of both systems in rats. Stability studies of selected ISI and ISM formulations were studied in order to determine the shelf life of the drug in both systems at 4°C.

MATERIALS AND METHODS

Materials

PLGA polymer (50:50) (intrinsic viscosity 0.5 dl/g, MW 60,000–70,000 Da) was purchased from Lactel Pharmaceuticals (Pelham, AL, USA). Montelukast sodium, NMP, triacetin, DMSO, ethyl acetate, 5-methyl 2-nitrophenol, sodium dihydrogen phosphate, sodium acetate trihydrate, acetonitrile, orthophosphoric acid, monobasic potassium phosphate, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Phosphate-buffered saline (PBS; pH 7.4) powder was purchased from Sigma Aldrich (Gaithersburg, MD, USA). Peanut oil was purchased from Spectrum Chemical (Gardena, CA, USA). Pluronic F68 NF was obtained from BASF Corporation (Mount Olive, NJ, USA). Aluminum monostearate was purchased from VWR (West Chester, PA, USA). Sprague Dawley rat plasma was purchased from Innovative Research, Inc. (Novi, MI, USA). All the above materials were of analytical grade or better.

Methods

Preparation of ISI Formulations

Polymeric solutions of 20%, 30%, and 40% (*w*/*v*) were prepared by dissolving PLGA in NMP, DMSO, triacetin, or ethyl acetate in a scintillation vial by shaking in an environmental shaker (VWR, West Chester, PA, USA). The mixture was shaken at room temperature for about 72 h until formation of a clear polymeric solution. The ISI formulations contained 10% (*w*/*v*) MK in the polymeric solution. Briefly, 300 mg of MK was added to a calibrated amber glass vial. The polymeric solution, about 3 mL measured by a 3-mL syringe, was added to the vial and mixed for about 24 h until the drug is completely dissolved in the polymeric solution. Finally, the volume was adjusted to the 3-mL calibration mark with the polymeric solution and mixed well.

Preparation of ISM

A polymeric solution of 30% PLGA in NMP or DMSO containing 10% (*w*/*v*) MK was prepared as described in the previous section. This polymeric phase was emulsified into an external peanut oil phase at three different polymer-to-oil phase ratios of 1:1, 1:2, and 1:4. The emulsification process was achieved by probe sonication using Branson Sonifier 250 (Branson Ultrasonic Corporation, Danbury, CT, USA) at output of 250 W and frequency of 20 KHz for 30 s under ice cooling. Pluronic F68 (1% (*w*/*w*), based on the amount of the total formulation) was dissolved in the polymer phase and aluminum monostearate (2% (*w*/*w*), based on peanut oil) in the oil phase to increase the stability of the emulsion (10).

In Vitro Release Study

One hundred milligrams of ISI and ISM formulations was added to 100 mL of PBS, pH 7.4 in a glass jar with a lid. A syringe fitted with a 20-gauge needle was used to add the formulation to the buffer. The glass jars were placed in an environmental shaker set at 37°C and 100 rpm. The clear Plexiglas front door of the shaker was completely covered with aluminum foils to avoid exposure of the glass jars to light. Aliquots of 1 mL were taken from each bottle at 1, 3, 5, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, and 60 days and analyzed for the drug. The dissolution medium was replaced with fresh medium to maintain a sink condition. The concentration of the drug in the collected samples was analyzed by UV spectrophotometer (DU 800, Beckman Coulter, Miami, FL, USA) at wavelength of 350 nm. Each experiment was run in triplicates.

Morphological Study of Formulations

The morphological properties of both ISI and ISM were investigated using scanning electron microscope (SEM; Philips XL30, Eindhoven, the Netherlands) operated at 4–25 kV. Samples of both systems were prepared by injecting known volume of each system into 100 mL of PBS and kept in an environmental shaker at 37°C for 24 h. The formed implants and microparticles were separated from the media and frozen at −80°C for 4 h. The frozen samples were then lyophilized for 24 h in a freeze drier (FreeZone 6, Labconco, USA). The dried samples were sputter-coated with gold, and SEM micrographs were obtained.

In Vivo Study

A total of 18 male Sprague Dawley rats (weight 200–224 g) were used in this study. Animals were maintained with a 12-h alternating light-and-dark cycle with free access to food. The rats were divided into three groups as follows: group I, 30% PLGA in NMP (ISI formulation); group II, 30% PLGA in NMP/oil (1:4) (ISM formulation); and group III, control

(saline, 0.1 mL). The formulations and saline were administered intramuscularly into the right musculus rectus of the rats (n=6 per group). The dose of MK administered was 30 mg/kg in the rats as a single injection for both the ISI and the ISM systems. The Institutional Animal Care and Use Committee (IACUC) approved the experimental procedures described in this study. All the procedures were in compliance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Blood samples were collected using the tail-vein bleeding (15). Blood samples (0.5 mL) were collected at 6, 12, 15, 18, 21, and 24 h and at days 3, 5, 7, 10, 14, 18, 21, 24, 28, and 31. The collected blood was centrifuged immediately at 4,300×g at 8°C for 15 min, and the separated plasma was frozen until analysis. The plasma concentration of MK was determined by using the high-performance liquid chromatography (HPLC) method described below. Pharmacokinetic parameters, such as maximum plasma concentration (C_{max}), time point of maximum plasma concentration (T_{max}), area under the plasma concentration–time curve from 0 h to the last measurable concentration (AUC_{0-t}), area under the plasma concentration–time curve from 0 h to infinity ($AUC_{0-\infty}$), mean residence time (MRT), and clearance, were determined using the pharmacokinetic software Kinetica ™ (version 4, Thermo Electron Corporation, Waltham, MA, USA).

After the *in vivo* study has been completed, the animals were euthanized with carbon dioxide and their intact skin along with intramuscular tissue was excised, and the injection sites for the ISI and ISM were compared to that of the control group. A sharp blade was used to make a cut section in the skin and intramuscular tissue to reach the site of injection. The tissue of the injection sites was visually examined for possible swelling, redness, or inflammation as previously reported (16).

Quantification of Montelukast in Plasma by HPLC

A modified HPLC method reported in the literature (17) was used for the quantification of MK. In the present study, 5-methyl 2-nitrophenol was used as the internal standard (I.S.) instead of mefenamic acid. Due to the light sensitivity of MK, stock solutions and calibration standards were kept in amber glass containers or protected from light by wrapping the tube in aluminum foil. Stock solutions of MK (5 μg/mL) and I.S. (5-methyl 2-nitro phenol, 5 μg/mL) were prepared separately in acetonitrile/methanol (70:30, *v*/*v*) and methanol, respectively. All stock solutions were stored at 4°C. Calibration curves with concentrations of 5, 10, 20, 50, 100, 500, and 1,000 ng/mL were prepared by dilution of aliquots of the stock solution with rat plasma. To 500 μL of plasma was added 500 μL of I.S. solution and 500 μL of acetonitrile in a screw-cap glass tube. The mixture was vortex-mixed for 60 s and centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was transferred to an autosampler vial, and 50 μL of it was injected into the HPLC system (Waters Corporation, Milford, MA, USA) using an Adsorbosphere C_8, 5 μm, 150× 4.6 mm column for the separation. The mobile phase was comprised of acetonitrile/25 mM acetate buffer at pH 4.0 (70:30, *v*/*v*). The 25-mM acetate buffer was prepared from sodium acetate trihydrate solution in water (3.4 g/L) adjusted to pH 4.0±0.1 with glacial acetic acid. The mobile phase was delivered through the column at a flow rate of 1.0 mL/min. The detector was set at a wavelength of 350 nm, the maximum wavelength of UV absorption of MK in the mobile phase used.

Stability Study

The formulations used in the *in vivo* study were incubated at 4°C, 25°C, and 40°C (18) to periodically measure drug content and pH. The pH was measured by immersing the electrode directly into the formula using an Accumet AR 60 pH meter (Fisher Scientific, Fair Lawn, NJ, USA). Drug content was assessed by taking aliquot from each samples, mixed with known volume of acetonitrile, vortexed, centrifuged at 1,100×g, filtered using 0.45 μm filter, and the resulting solution was analyzed by HPLC utilizing the chromatographic conditions described above. For the ISM system, only the acetonitrile part (at the top) was used for the drug analysis while the peanut oil part was separated at the bottom. The kinetic order for the degradation and shelf life (t_{90}, the time when 10% of the drug is degraded) of the drug in both formulations was determined over 12 months of study at 4°C.

RESULTS AND DISCUSSION

Preparation of ISI Formulations

Poly-lactic-co-glycolic acid was used as the polymer as it has the regulatory approval for parenteral application in human (19) and good biodegradability, biocompatibility, and mechanical strength (20). Four solvents were chosen for ISI formulations: NMP, DMSO, triacetin, and ethyl acetate. PLGA 50:50 in a concentration of 20%, 30%, and 40% (*w*/*v*) was selected while the drug concentration in all the preparations was constant (10%, *w*/*v*). Table I represents the composition of the prepared

Table I. *In Situ* Implant and *In Situ* Microparticles of Montelukast

	PLGA 50:50 (*w*/*v*)	Solvent	Polymer phase/oil phase
ISI formulation			
MK1	20%	NMP	
MK2	30%	NMP	
MK3	40%	NMP	
MK4	20%	DMSO	
MK5	30%	DMSO	
MK6	40%	DMSO	
MK7	20%	Ethyl acetate	
MK8	30%	Ethyl acetate	
MK9	40%	Ethyl acetate	
MK10	20%	Triacetin	
MK11	30%	Triacetin	
MK12	40%	Triacetin	
ISM formulation			
MK13	30%	NMP	1:1
MK14	30%	NMP	1:2
MK15	30%	NMP	1:4
MK16	30%	DMSO	1:1
MK17	30%	DMSO	1:2
MK18	30%	DMSO	1:4

ISI in situ implant, *ISM in situ* microparticles, *PLGA* poly-lactic-co-glycolic acid, *MK* montelukast, *NMP N*-methyl pyrrolidone, *DMSO* dimethyl sulfoxide

ISI MK formulations. The selection of the studied solvents was based on their safety, biocompatibility, stability of PLGA, and miscibility with water. NMP and DMSO have been used in commercial injectable products for human use (21). Triacetin is used in oral dosage forms and is generally recognized as safe (FDA's GRAS list). It has been considered as a potential parenteral nutrient (22). Ethyl acetate is an International Conference on Harmonization (ICH) Class 3 solvent which may be regarded as less toxic and of low risk to human health (21). Ethyl acetate has been used in the preparation of PLGA microspheres for parenteral preparation (23). With regard to the stability of PLGA, a faster degradation occurred in polar protic solvents (2-pyrrolidone, PEG 400, triethyl citrate) than in polar aprotic solvents (*N*-methyl-2-pyrrolidone, DMSO, triacetin, ethyl acetate) (21). These solvents span the range from a relatively strong solvent with high water miscibility (NMP and DMSO) to solvents of lesser power, having progressively lower water miscibility (triacetin and ethyl acetate). In addition, NMP, DMSO, triacetin, and ethyl acetate were chosen because they have an LD_{50} of higher than 2 mL/kg which is considered to be safe for use in injectable implant systems (10,24).

In Vitro Release Study of the ISI Formulations

Figure 1 illustrates the release of MK from different PLGA concentrations in NMP and DMSO. Both systems, visually, seem to have a tri-phasic release pattern. Drug release from PLGA-based drug delivery systems is sometimes bi-phasic, but the tri-phasic profile is probably the most common (19). The large PLGA particles or drug delivery systems often exhibit tri-phasic release pattern (25) while small PLGA particles and particles coated with a thin PLGA film often exhibit a bi-phasic release profile with a relatively rapid second phase (26). After the initial burst in drug release, a diffusion-controlled slower release phase follows. Finally, a period of faster release often attributed to the onset of erosion where the molecular weight of PLGA approaches a certain lower threshold (27).

The initial burst in the drug release (during the first few hours of administration) is a major problem associated with ISI system. The burst effect may be due to the time elapsed between the administration and formation of the implant. The possible explanation for the burst effect is also related to the release of drug adsorbed on the surface of polymeric matrix (28), unequal distribution of the drug inside the polymeric matrix network (29,30), and/or rapid dissemination of the drug to the surrounding medium during the solidification process (31). The burst release can be controlled by factors such as molecular weight and concentration of the polymer, the solvent, and other additives used in the ISI system (32). The drug dissolved in the polymeric solution may precipitate outside the PLGA matrix during the formation of the *in situ* implant. The precipitation may happen more to hydrophobic drugs. Since montelukast sodium is a water-soluble drug (33), we do not anticipate the precipitation of the drug outside the PLGA matrix during the *in vitro* release study.

Polymer concentration of 20%, 30%, and 40% showed an initial drug release during the first 24 h of 23%, 20%, and 16%, respectively, for NMP and 24%, 22%, and 19%, respectively, for the same polymer concentrations with DMSO. It is important to mention that as the concentration of the polymer increased from 20% to 40%, the drug release decreased. Drug release continued up to 24, 28, and 38 days for 20%, 30%, and 40% PLGA, respectively, in NMP and 21, 24, and 35 days for the same polymer concentrations in DMSO. In general, the release of MK from NMP was slightly slower than that from DMSO. This may be due to the difference in the solvating power of the two solvents.

The results obtained for the release of MK from formulations containing ethyl acetate and triacetin are shown in Fig. 2. The *in vitro* release of MK from both ethyl acetate and triacetin is seen to be considerably lower than that of NMP and DMSO. As previously noticed with NMP and DMSO, drug release from ISI system containing ethyl acetate and triacetin also decreased with increased concentration of polymer. A concentration of 20%, 30%, and 40% PLGA in ethyl acetate showed an initial release of 11%, 10%, and 9%, respectively, within the first 24 h and 10%, 9%, and 7%, respectively, from triacetin containing formulations. The results also revealed that PLGA-based ISI in ethyl acetate released 96%, 91%, and 87% of MK, respectively, within 60 days while it was 92%, 86%, and 82%, respectively, with triacetin.

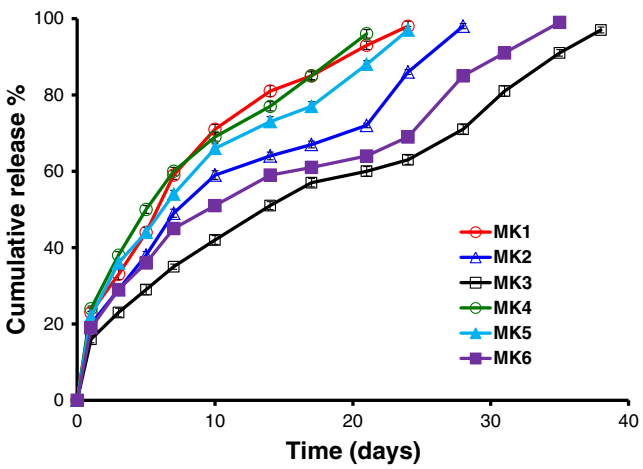

Fig. 1. *In vitro* release of montelukast (mean±SD, $n=3$) from *in situ* implant systems containing PLGA in NMP and DMSO

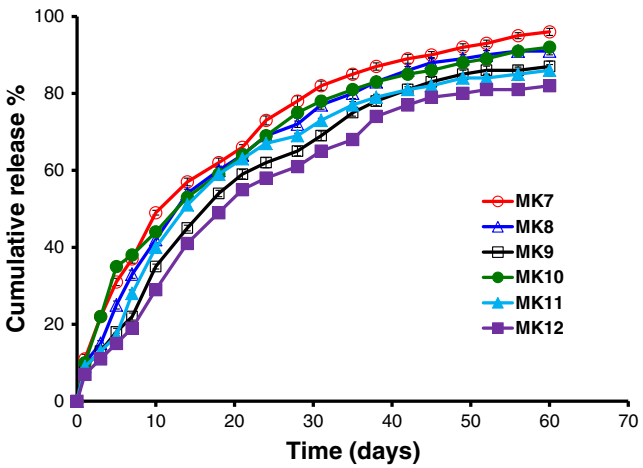

Fig. 2. *In vitro* release of montelukast (mean±SD, $n=3$) from *in situ* implant systems containing PLGA in ethyl acetate and triacetin

The release of MK from the partially miscible solvents ethyl acetate and triacetin is much slower than that from the miscible ones, and this could be attributed to the solubility and plasticizing effect of these solvents. The solubility of triacetin in water is 77.8 mg/mL (34), while ethyl acetate has solubility of 8.7% (*v*/*v*) in water (35). Both solvents due to their finite solubility in water may have slowed solvent exchange with the buffer and may have led to minimize pore formation in the formed implant and slowed the drug release rate. Triacetin and ethyl acetate also plasticized the PLGA polymer and lowered the burst effect and extended the release pattern (34). Drug release from ISI prepared with miscible solvents (PLGA in NMP or DMSO) follows the dynamics of phase inversion (process of precipitation of the polymer from solvent/nonsolvent system; changing from liquid phase to solid phase) (36,37): a burst followed by a slower release. On the other hand, ISI prepared with partially water-miscible solvents (PLGA in triacetin or ethyl acetate) exhibit much slower phase inversion that resulted in a more uniform drug release (36).

The type of solvent and PLGA concentration had a pronounced effect on the *in vitro* drug release from ISI system. Solvents that are relatively miscible with the aqueous body fluids promote rapid migration of water into the polymer composition, resulting in a burst effect, while solvents with lower affinity for water significantly reduced the water uptake and resulted in slower release characteristics as compared to implants made from miscible solvents.

Montelukast sodium is a hydrophilic drug with a solubility of 100±0.16 g/l in water (33). Previous studies have suggested the suitability of water-soluble drugs such as metoclopramide monohydrochloride from PLGA/benzyl benzoate *in situ* gelling preparation (38). The drug release profile, as defined by the time required for 100% release and the steady-state rate, varies significantly with the type of drug incorporated in the PLGA matrix (39). Hydrophilic drugs seem to have faster release rate compared to the hydrophobic ones. The times for complete release of aspirin (water solubility, 4.99 mg/mL) and haloperidol (water solubility 0.13 mg/mL) from PLGA/drug pellets were 13 and 38 days, respectively (39). Our finding indicated a higher and faster release rate for MK which has much higher solubility in water compared to the mentioned drugs.

Preparation of ISM Formulations

The key parameter for the preparation of microparticles is the use of partial water miscibility of the organic solvents. NMP and DMSO are slightly miscible with oil and form two phases(organic/oil) systems (40). The ISM systems prepared with miscible solvents showed good syringeability and injectability and produced a stable ISM system when compared to that prepared with partially miscible ones.

Table I showed the composition of ISM formulations of MK (MK13-18). Water-miscible solvents such as NMP and DMSO have been used for the preparation of ISM system (41). ISM system prepared with biocompatible peanut oil as the external phase has much lower myotoxicity than that of the ISI system (42).

In Vitro Release Study of ISM Formulations

Figure 3 illustrates the release of MK from different PLGA concentrations in the ISM systems prepared with NMP and DMSO. ISM formulations prepared with NMP and at different polymer-to-oil phase ratios: MK13, MK14, MK15 had drug release in the first 24 h of 15%, 14%, and 12%, respectively. The corresponding ISI preparation, MK2, had 20% drug release in the first 24 h. The ISM formulations prepared with DMSO: MK16, MK17, and MK18 showed 17%, 15%, and 14% drug release, respectively, during the first 24 h. The corresponding ISI preparation, MK5, had 22% drug release in 24 h. From these data, it is clear that the ISM formulations reduced the initial burst release of MK as compared to the ISI formulations. Also, the ISM formulations extended the release of MK. Both these two effects may be attributed to the external oil phase that acts as a barrier for the solvent. As the volume of external oil phase increased, the initial solvent diffusion rate may have decreased. The low solubility of the drug in the external oil phase may have caused the drug to stay in the inner polymer phase as it was enclosed within the precipitated microparticles. The release of MK from 30% PLGA in DMSO was higher than that for 30% PLGA in NMP as observed with previous studies with ISM systems (41). The ISM system maintained the same tri-phasic *in vitro* drug release pattern as seen with the ISI, but the initial burst release of the drug was decreased and the release was prolonged compared to that of the ISI system.

Morphological Study of ISI and ISM

The morphological properties of the *in situ* implants and microparticles were determined by SEM (Fig. 4). The *in situ* implant which was collected 24 h after incubation in the buffer medium had a porous surface (Fig. 4a), which accounts for the rapid initial drug release. The solvent used in the preparation of the ISI has an effect on the morphology of the ISI. Water-miscible solvents lead to porous surface due to rapid exchange between these solvents and the surrounding aqueous media. In earlier studies, ISI prepared with water-miscible solvents,

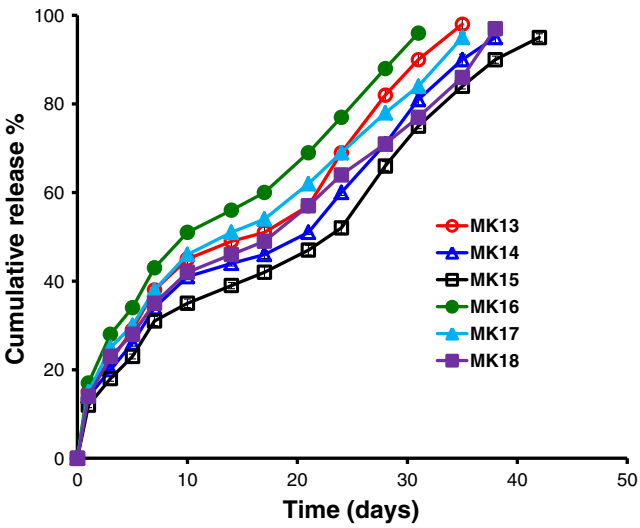

Fig. 3. *In vitro* release of montelukast (mean±SD, $n=3$) from *in situ* microparticle systems containing PLGA in NMP and DMSO

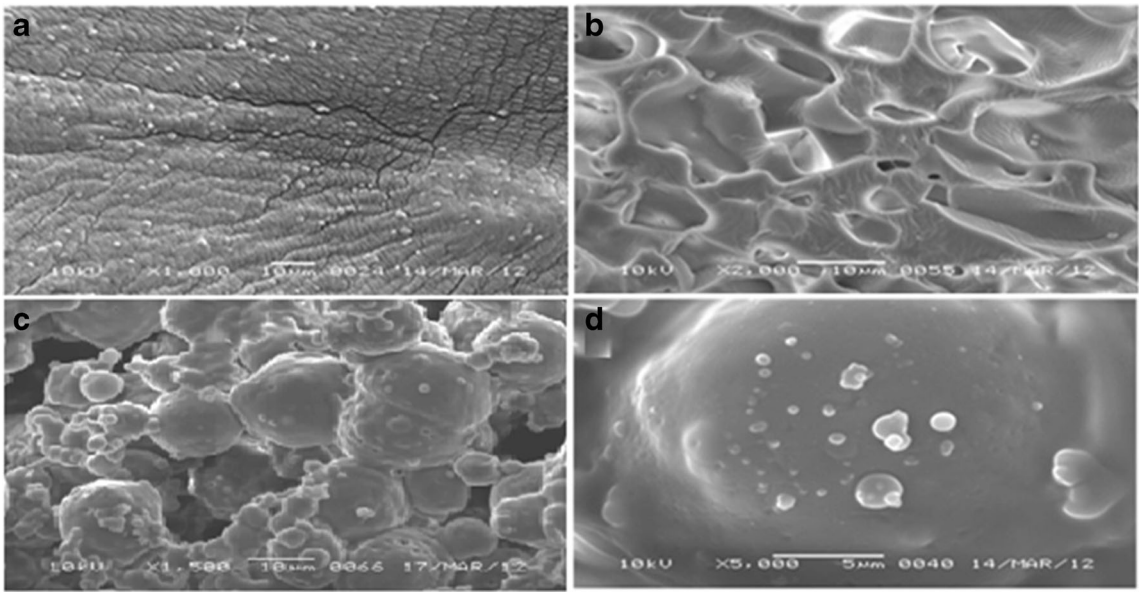

Fig. 4. Scanning electron microscopic images of ISI and ISM of montelukast; **a** surface of MK2 implant; **b** cross section of MK2 implant; **c**, **d** surface of MK15 microparticles

such as NMP, DMSO, and 2-pyrrolidone, lead to irregular porous surface due to fast solvent release in the PBS buffer (41). This also explains the high initial drug release from ISI systems prepared with these solvents. ISI system prepared with solvents having lower affinity for water such as triacetin may reduce the solvent exchange process during the implant formation and may form less porous implants.

The ISM had a less porous surface (Fig. 4c, d), which explains the lower initial burst of the ISM system. The ISM systems were made utilizing ISI systems which were initially prepared with water-miscible solvents, NMP and DMSO. The internal polymer phase of the ISM system precipitates when it comes in contact with the aqueous media, but due to the external hydrophobic oil phase, the solvent diffusion rate between the ISM system and the buffer media is slower than that of the ISI system (41). This slow solvent exchange process has led to less porous microparticles and slower initial drug release.

HPLC Method for Quantification of Montelukast in Plasma

The I.S. and MK were well separated by the HPLC method. The analytical method demonstrated excellent chromatographic specificity and selectivity with no endogenous plasma interference at the retention times of MK and the internal standard. The plasma calibration curve was found to obey Beer's law within the range 5–1,000 ng/ml in which the correlation coefficient (r) was 0.9998 (n=3).

Pharmacokinetic Study

Plasma concentration of MK was determined following intramuscular injection of ISI formulation of MK, 30% PLGA in NMP (MK2), and its corresponding ISM formulation prepared with a 30% polymer in NMP/oil phase ratio of 1:4 (MK15). MK2 was selected as it illustrated a better drug release for 1 month and its acceptable syringeability and ease of administration. The corresponding ISM formulation, MK15, was selected to compare the *in vivo* performances of both ISI and ISM systems utilizing the same polymer, polymer concentration, and solvent. Polymer-to-oil ratio of 1:4 was selected as it demonstrated much lower initial drug burst and extended release compared to the other polymer-to-oil phase ratios.

Each rat was administered 30 mg/kg of MK (approximately 7 mg) as a single injection for the 1-month study period. The dose was calculated based on the daily adult dose (10 mg) in human, consideration of 5–7-fold higher metabolic activity of rats than the human (43), and surface area ratios of human and rat (44). Both ISI and ISM formulations had good syringeability and injectability during their intramuscular administration to the rats with 20-gauge needle.

Figure 5 illustrates the mean plasma level *versus* time following the intramuscular injection of ISI and ISM systems. The C_{max} of MK was 2.43±0.11 µg/mL, 15 h after injection of

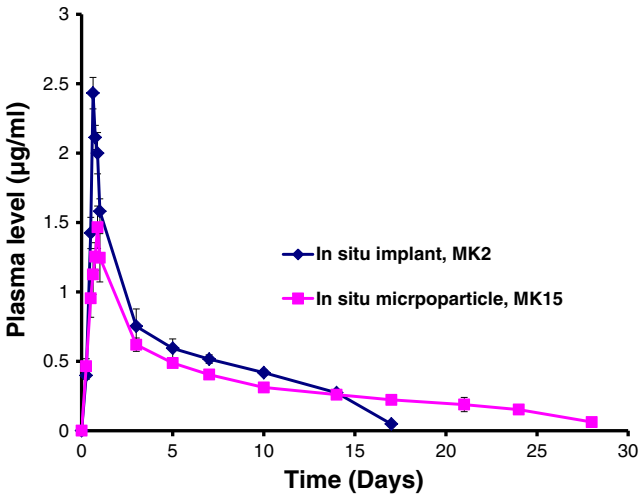

Fig. 5. Plasma profile of montelukast (mean±SD, n=6) following intramuscular injection of ISI and ISM formulations of montelukast in rats

the ISI formulation, and C_{max} of MK was 1.47±0.15 μg/mL, 21 h after injection of the ISM formulation. These results indicate that ISM formulation resulted in a slower release of MK compared to the ISI formulation, consistent with the *in vitro* release. Montelukast exhibits two compartment pharmacokinetics like zafirlukast, but MK is more potent inhibitor of leukotriene (45). Montelukast sodium is characterized by a good safety margin. It could be administered in rats in a dose up to 5 mg/kg/day for the treatment of acute hepatopathy induced by carbon tetrachloride (CCl_4) (46). The C_{max} obtained for both ISI and ISM systems were well tolerated by the rats. On day 3, the mean plasma concentration of the drug was 752 ng/mL and declined to 48 ng/mL on day 17 in the ISI-treated group. A longer sustained release of MK in plasma from ISI system may be obtained by using MK3 (40% PLGA in NMP). In the ISM-treated group, on day 3 the mean plasma concentration was 619 ng/mL and declined to 62 ng/mL on day 28. These plasma levels are within the therapeutic range obtained after a single oral dose of MK (12). The drug loading was chosen to be 10% (*w*/*v*) of the polymer phase in both ISI and ISM systems. Increased drug loading may be used in future studies to reduce the amount of the formulation needed for injection.

The $AUC_{0-\alpha}$, which reflects the total amount of active drug which reaches the systematic circulation, was found to be 230 and 243 μg h/mL for ISI and ISM, respectively. Although the AUC of both formulations are similar, the lower C_{max} and longer sustained release of the drug in plasma from the ISM system make it advantageous over the ISI formulation. The $AUMC_{0-\alpha}$ (area under the concentration times time *versus* time curve, zero to infinity) was found to be 31,968 and 60,323 μg h^2/mL for ISI and ISM, respectively, while the MRT was 139 and 248 h for ISI and ISM, respectively, in the rat. The total clearance of MK in the rat was found to be 0.506 and 0.474 mL/min for ISI and ISM formulations, respectively. The half-life, mean residence time, and clearance of MK in the human following a 7-mg intravenous administration of montelukast sodium were 6.7 h, 5.4 h, and 30.8 mL/min, respectively (14). The ISI and ISM formulations, providing sustained release of MK, will have better advantage than administering the drug itself.

Control groups treated with normal saline were used to compare the effect of the ISI and ISM formulations at the injection site and their biocompatibility. The tissues in treated group showed no marked difference in appearance from that in the control group, which confirmed the biocompatibility of the intramuscularly injected formulations. Sharma *et al.* (16) administered ellagic acid and ellagic acid-loaded PLGA nanoparticles subcutaneously in rats from biodegradable *in situ* gelling system and found no capillary proliferation, fibroblast formation, monocyte infiltration, and inflammation upon administering these formulations.

Stability Study

In order to establish a recommended storage condition for both systems, the formulations used in the *in vivo* study were incubated at different temperatures to select the most appropriate condition. The pH and drug content were determined under three different temperatures 4°C, 25°C, and 40°C with their relative humidity for 3 months. After 90 days of study, at 4°C or even 25°C, drug content was around 98%. However, increasing the temperature from 25°C to 40°C resulted in marked decrease in the drug content of the formulations. The results for the drug content were also associated with changes in pH. Measuring the pH of the formulations was done to evaluate the drug/polymer degradation. The formulations were liquid and prepared with water-miscible solvent (NMP). Measurement of pH in the non-aqueous system may not be reliable. Calibration of the pH meter using standard (aqueous) buffers may cause discrepancy in pH readings of non-aqueous solutions. The NMP used is hygroscopic, contained about 0.5% water, and has a pH around 8.0 (product description; material safety data sheet). The polymer, PLGA, is biodegradable and may have some hydrolysis product such as lactic and glycolic acid. Thus, whatever small amount it may be, it is possible to have H^+ present in the formulation. The pH was monitored to follow any change in the system rather than the absolute pH of the system. The physical characters of both formulations did not change significantly; both were stable, no color changes were noticed, and both solidified in the buffer upon injection.

Table II. Montelukast Content and pH of ISI (MK2) and ISM (MK15) Formulations During 1 Year Storage at 4°C

	Montelukast content (%, *w*/*v*)		Formulation pH	
Formulation	ISI	ISM	ISI	ISM
0 month	100	100	8.91	8.36
2 months	99.75	99.55	8.89	8.33
4 months	99.25	99.35	8.87	8.29
6 months	98.40	98.65	8.84	8.27
8 months	97.65	97.97	8.82	8.26
10 months	96.60	97.20	8.20	8.24
12 months	95.90	96.50	8.19	8.22

(n=3, SD <5% of the means)
ISI in situ implant, *ISM in situ* microparticles

The drug content and pH of both systems were determined for a period of up to 12 months; data are presented in Table II. There was no significant degradation of MK for 12 months after storage at 4°C, and both systems are suitable for drug formulation.

Kinetic parameters for stability studies of MK formulations revealed that the degradation of MK seems to be zero-order reaction (correlation coefficient of 0.99627 and 0.99069 for MK2 and MK15, respectively, for zero order *versus* −0.99596 and −0.99033, respectively, for first order). The t_{90}, which is a direct interpretation of the length of time through which each formula would remain and comply the official requirements of drug contents, were calculated to be 2.31 and 2.81 years for MK2 and MK15, respectively.

CONCLUSION

The *in vitro* release rate of MK in the ISI system decreased as the concentration of the polymer increased. Implants prepared with ethyl acetate and triacetin exhibited longer drug release profile compared to those made from NMP and DMSO. The ISM system showed lower initial burst and slower release of MK than ISI. The pharmacokinetic data

demonstrated sustained level of MK in plasma for more than 17 days (range 2.4–0.05 μg/mL) in ISI-treated group and more than 28 days (range 1.5–0.06 μg/mL) in ISM-treated group. The ISM formulation of MK provided lower initial burst and longer sustained release of the drug as compared to the ISI formulation. The ISM formulation (polymer phase: 30% PLGA in NMP; polymer/oil phase ratio of 1:4) can be used to formulate 1-month sustained release of MK for intramuscular administration. After 12 months of storage at 4°C, more than 95% of MK was intact in both ISI and ISM formulations used in this study.

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