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Research Paper

In Vitro and *In Vivo* Drug Release from a Novel *In Situ* Forming Drug Delivery System

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Purpose. The objective of this work was to investigate the influence of various preparation and formulation parameters on the *in vitro* and *in vivo* release of bupivacaine hydrochloride from an injectable *in situ* forming microparticle system (ISM).
Methods. The *in vitro* drug release of ISM was investigated as a function of various formulation and process parameters and was compared to the drug release from *in situ* forming implants and conventional microparticles. *In vivo* studies were carried out in male Sprague–Dawley rats.
Results. Upon contact with an aqueous medium, the internal polymer phase of the ISM system solidified and formed microparticles. The initial drug release from ISM systems was reduced with decreasing polymer phase/external oil phase ratio. An advantage of the ISM system compared to *in situ* implant systems was the significantly reduced burst effect, resulting in drug release profiles comparable to microparticles prepared by conventional methods. The *in vivo* drug release studies were in good agreement with the *in vitro* drug release. With the ISM system, the analgesic effect of the bupivacaine hydrochloride was prolonged when compared to the injection of a drug solution or drug-polymer solution.
Conclusions. ISM are an attractive alternative for parenteral drug delivery systems.

KEY WORDS: biodegradable polymers; *in situ*; local anesthetic; microparticles; sustained-release depot.

INTRODUCTION

Local anesthetic blockade of nerves is a mainstay in the management of many forms of acute and chronic pain. Following a single injection, currently available local anesthetics rarely provide analgesia for longer than 4–6 h (1). Infusion pumps with indwelling catheters are possible alternatives. However, these catheter infusions are awkward or difficult to secure in many locations in the body. Nerve blocks with phenol, alcohol, heat, or cryoprobes produce localized tissue destruction, unwanted deficits, and new forms of pain (2).

Application of a timed-release local anesthetic preparation adjacent to nerves was shown to provide prolonged regional anesthesia of peripheral nerves in patients with postoperative pain, cancer, nerve injuries, chronic orofacial pain, or other conditions requiring chronic pain management (1). In general, the regional administration of local anesthetic drugs should be improved by the development of a drug delivery system leading to sustained release of the drug at the site of application while reducing systemic drug levels. This is particularly important with the highly potent but cardiovascular toxic bupivacaine hydrochloride (3).

Several approaches have been investigated in order to control the regional administration of local anesthetics including: crystallization by modified ionization (4), liquid–solid emulsion gel (5), use of lipid carriers (6) or liposomes (7). The feasibility of prolonged regional blockade using polymers was shown with surgically implantable pellets (1,8) and injectable microparticle formulations (3,9–11). The implantable pellets were prepared with bupivacaine hydrochloride in a polyanhydride matrix (1,8). Depending on the administered dose sensory and motor block blockade lasted for 2–6 days. However, the administration requires surgical implantation of the pellets.

Injectable bupivacaine or tetracaine loaded poly(D,L-lactide)(PLA) or poly(D,L-lactide-co-glycolide) (PLGA) microparticles have been prepared by the solvent evaporation/extraction method (3,9,10). Depending on the administered dose and additives (dexamethasone seems to prolong the effect of the local anesthetics by an unknown mechanism), the localized effects ranged for up to 5.5 days (3). The prolonged blockade of peripheral nerves was shown to be feasible without any systemic side effects with these microparticle formulations.

Nevertheless, the preparation of implants, and especially of microparticles, is based on complicated, multiple step processes with many process and formulation parameters that need to be controlled during the preparation (11). As an

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alternative to solid implant or microparticle formulations, liquid drug-polymer formulations have been developed, which form implants *in situ* upon injection when placed in contact with body fluids through the precipitation of the polymer (12–14). PLA- or PLGA-polymers are dissolved in water-miscible solvents, such as *N*-methyl-2-pyrrolidone (NMP) or dimethylsulfoxide (DMSO). The polymer solidifies at the site of injection and forms an implant upon injection of the drug containing polymer solution. This technology has been utilized for low and high molecular weight (M_w) drugs (15–17). A commercially available product is Atridox® used for the periodontal delivery of doxycycline (18). The disadvantages of these *in situ* implant systems (polymer solutions) are the initial rapid release prior to solidification of the polymer and the difficult injectability of the highly viscous polymer solution (19,20).

As an alternative to microparticles or *in situ* implant systems a novel *in situ* forming microparticle system (ISM) has been developed (19,21). This ISM system consists of an internal, drug containing polymer-solvent phase (polymer phase) emulsified into an external phase (an oil phase more compatible with tissue). Upon injection of this emulsion, the internal polymer phase releases the drug in a controlled release fashion. Solvents for the polymers are NMP, DMSO and 2-pyrrolidone, which are able to form highly concentrated polymer solutions. Peanut oil, an oil for injection, can be chosen as a biocompatible external oil phase. The ISM systems have significantly reduced myotoxicity and present a lower viscosity (the viscosity is primarily controlled by the external oil phase and not by the internal polymer phase). Therefore, they are easier to inject when compared to the polymer solutions (19,20). In addition, the preparation process for ISM is simple when compared with classical techniques for the preparation of microparticles. Fatty oils are commonly used for parenteral drug delivery (e.g. for the delivery of hormones). Nevertheless, their suitability needs to be evaluated case by case. Particularly antigenic side effects might occur when using fatty oils for administration of peptides.

The objective of this study was to investigate the influence of various formulation and preparation parameters on the *in vitro* and *in vivo* bupivacaine hydrochloride release from an injectable *in situ* forming microparticle system (ISM). A hot plate model was used to quantify the analgesic effect of the bupivacaine release from *in situ* microparticles and implants following intramuscular (i.m.) injection in rodents.

EXPERIMENTAL SECTION

Materials

The following chemicals were obtained from commercial suppliers and used as received: poly(D,L-lactide-co-glycolide) (PLGA, RG 503, M_W 42,800, Boehringer Ingelheim, Ingelheim, Germany), bupivacaine hydrochloride (Sigma Aldrich Company, St. Louis, USA), acetone, acetonitrile, ethanol, ethyl acetate, heptane, methanol, methylene chloride, potassium dihydrogen phosphate, 0.05 M sulfuric acid (H_2SO_4), sodium acetate, sodium hydroxide (Merck, Darmstadt, Germany), 2-pyrrolidone (Soluphor®), Pluronic F 68 (BASF AG, Ludwigshafen, Germany), peanut oil (Henry Lamotte GmbH, Bremen, Germany), aluminum-monostearate (Fluka Chemie AG, Buchs, Swiss), polyvinyl alcohol (PVA, Mowiol 40-88, Clariant GmbH, Frankfurt, Germany), sodium pentobarbital, 0.9% NaCl solution (normal saline, Abott Laboratories, Chicago, USA). All chemicals were reagent grade or higher.

Methods

Preparation of the in situ forming drug delivery systems. In situ implants were prepared by mixing PLGA and bupivacaine hydrochloride with 2-pyrrolidone in glass vials until the formation of a clear solution. The concentration of polymer and drug were kept constant on a level of 40% (*w*/*w*, based on solvent and polymer) and 10% (*w*/*w*, based on polymer), respectively.

The ISM systems were prepared by emulsifying the drug-containing polymer solutions (PLGA in 2-pyrrolidone) (polymer phase) into a peanut oil phase (oil phase) at a polymer to oil phase ratio of 1:1, 0.5:1, 0.25:1 and 0.1:1. Ready-to-inject formulations were prepared by probe sonication at 40 W (Bandelin Sonopuls HD 200, Bandelin electronic, Berlin, Germany) for 30 s or Ultra-Turrax mixing at 12,500 rpm (IKA-Labortechnik, Staufen, Germany) for 60 s under ice cooling. Alternatively, emulsion formation prior to injection was carried out using two syringes (2 ml glass syringes, Hypak, Becton Dickinson & Co., Franklin Lakes, USA) with the polymer phase in the first and the peanut oil phase in the second container. Prior to use, both syringes were connected to each other using a polypropylene connector (Becton Dickinson & Co., Franklin Lakes, USA) with an inner diameter of 1.5 mm. Emulsion formation was then carried out by hand mixing (50 mixing cycles). Pluronic F 68 (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 the peanut oil) in the oil phase to increase the stability of the emulsions. ISM formulations were prepared by Ultra-sonication if not otherwise mentioned.

Preparation of microparticles by film grinding. The active agent (10% *w*/*w* bupivacaine hydrochloride, based on the polymer) was dissolved in ethanol (0.5 ml) and added to a solution of PLGA in acetone (350 mg RG 503 in 6.5 ml acetone). The films were prepared by casting the drug containing polymer solutions into Teflon molds (5.7 cm in diameter). Dried films were removed from the Teflon surface, cut into 4×4 cm^2 test sections, and stored for 48 h in a desiccator prior to further experimentation. Microparticles were then prepared by grinding the drug-loaded films in a ball mill (Retsch Schwingmühle MM 2000, Retsch, Haan, Germany) cooled with liquid nitrogen. The microparticles were sieved and the size range of 50–100 μm was used for further experimentation.

Preparation of microparticles by the solvent evaporation method (W/O/W). An aqueous solution of bupivacaine hydrochloride (30 mg drug, 1 ml 0.01 M phosphate buffer pH 4.0) was emulsified with a sonicator (Bandelin Sonopuls HD 200, Bandelin Electronic, Berlin, Germany) into a solution of PLGA (300 mg RG 503) in methylene chloride (12 g) to form a W/O emulsion (sonication time=2 min). The primary emulsion was then added to the external aqueous

phase (800 ml water, 0.25% *w/w* PVA, 0.25 mol/l sodium chloride) with a propeller stirrer (1,500 rpm, Heidolph Elektro KG, Kelheim, Germany) to form the microspheres. The microspheres were collected, rinsed with water and sieved into various particle size fractions. Microparticles in the size range of 50–100 μm were used for further experiments.

In vitro drug release studies. ISM systems, *in situ* implants and microparticles were placed into dialysis bags (Medicell International Ltd., London, England; M_W cutoff 12–14,000 Da), one-site opened conical vials (material: polypropylene; length, 40 mm; maximum diameter, 8 mm, neoLab, Heidelberg, Germany) or injected directly into the release medium (n=3). The formulations were placed into 50 ml 0.01 M phosphate buffer pH 4.0 (0.05% *w/v* sodium azide were added as a preservative) at 37°C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). At predetermined time intervals, 2 ml samples (which were replaced with fresh medium) were withdrawn and assayed. After 48 h the complete medium was withdrawn and replaced by fresh medium at each sampling point. The bupivacaine hydrochloride content was measured with a computer connected Shimadzu-HPLC system (SCL-10 A System Controller, LC-10 A pump, DGU-3 A degasser, SIL-10 A auto injector, SDS-10AV UV-detector, Class-LC 10 software, Shimadzu, Kyoto, Japan). A 40 μl volume was injected onto a LiChrospher-100 RP 18.5 μm vertex column (Knauer GmbH, Berlin, Germany) using as the mobile phase a mixture of 60 ml acetonitrile and 40 ml of 0.01 M phosphate buffer pH 4.00; flow rate, 1.0 ml/min; 40°C; UV-detection at 220 nm.

Solubility of the drugs. Excess amount of bupivacaine hydrochloride was placed in 1 ml 0.01 M phosphate buffer pH 4.0 (n=3). The samples were shaken for 48 h at 37°C. The saturated drug solutions were filtered and then assayed as described above after appropriate dilution.

Scanning Electron Microscopy (SEM). The dried *in situ* microparticles or implants were coated for 70 s under an argon atmosphere with gold-palladium (SCD 040, Balzers Union, Lichtenstein) and then observed with a scanning electron microscope (PW 6703/SEM 515, Philips, Eindhoven, Netherlands).

Particle size and particle size distribution. Size distribution of the ISM was evaluated by dispersing about 1 g of the ISM emulsion into 50 ml 0.01 M phosphate buffer pH 4.0 containing 0.1% *w/w* Tween 80. For particle hardening the ISM were shaken for approximately 2 h in a horizontal shaker at 37°C (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). The ISM were collected using dialysis bags that were also used for drug release studies and rinsed with water. The particle size distribution of the *in situ* microparticles was determined by laser diffraction measurements (Coulter LS 230, Coulter Corporation, Hialeah, USA).

In vivo drug release studies. Sterile silastic cannulas were implanted into the right atria of male Sprague–Dawley rats (250–300 g) following the injection of an anesthetic cocktail as previously reported (22). The cannulas were filled with heparinized (100 U/ml) normal saline solution to prevent blood clotting and maintain cannula access. The rats were given 7 days to recover prior to the study. Following i.m. injection of the polymer solution (40% PLGA in 2-pyrrolidone) and the ISM system (polymer phase: 40% PLGA in 2-pyrrolidone; polymer/oil phase ratio of 0.25:1) into the right musculus rectus, blood samples (0.5 ml) were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h. The dose of bupivacaine hydrochloride administered was 5 mg for both the polymer solution and the ISM system. The blood samples were centrifuged immediately after collection and plasma was stored at −20°C for analysis of bupivacaine hydrochloride. Red blood cells were reconstituted in heparinized (40 U/ml) normal saline solution (0.20 ml) and reinjected into the rat to maintain blood volume. Heparinized (100 U/ml) normal saline solution was used to fill the catheter between periods of sample collection. All experiments were conducted in triplicate.

The bupivacaine plasma concentrations were determined after a modified method reported previously (23). A plasma sample (100 μl) was alkalinized with sodium hydroxide (100 μl, 1 M). Then, 3 ml of a heptane–ethyl acetate mixture (90:10, *v/v*) were added, the tube shaken for 30 min and centrifuged at 1,000 U/min for 10 min at 20°C. An aliquot (2.5 ml) of the organic phase was transferred to a tube containing 100 μl of 0.05 M H_2SO_4. After shaking for 30 min, the tube was centrifuged at 1,000 U/min for 10 min at 20°C and the organic phase discarded. The aqueous acid phase was buffered at pH 4.0 by transfer in a tube containing a methanolic solution of sodium acetate (50 μl, 0.2 M) that had been previously evaporated. The drug content was then measured with the Shimadzu-HPLC system (sample volume 40 μl, UV-detection at 205 nm). Analyses were performed with a LiChrospher-100 RP 18,5 μm vertex column (Knauer GmbH, Berlin, Germany) using as the mobile phase an acetonitrile/0.01 M phosphate buffer pH 4.0 mixture (40:60, *v/v*) at a flow rate of 1 ml/min. Plasma samples of known bupivacaine hydrochloride concentrations (0.1–2 μg/ml) were used to generate calibration curves.

Analgesia testing. The analgesic effect of bupivacaine hydrochloride was tested utilizing a hot plate at 54.0±0.5°C as reported earlier (24; n=3). Before any drug administration, the rats were habituated to the test for 5 days to obtain a stable control response value. On the study day, the rats were pre-tested three times on the hot plate. The values from the last two pretests were averaged and taken as baseline latency (BL). Post-drug latency (DL) was determined at 10 min intervals for up to 90 min following i.m. injection of the drug solution in 2-pyrrolidone. The 40% PLGA solution in 2-pyrrolidone and the ISM systems (40% PLGA in 2-pyrrolidone; polymer/oil phase ratio, 0.5:1 or 0.25:1) were tested after 15, 30, 60, 120, 180, 240, 300 min, 6, 24, 48 and 72 h. The dose of bupivacaine hydrochloride administered was 1 mg, 3 mg or 5 mg. The analgesic effect was calculated as the percentage change in reaction time from baseline latency values by the use of the formula, lick latency=$[(DL-BL)/BL]\times 100\%$.

Protocols for the *in vivo* study were approved by the Animal Care and Use Committee at the University in Florida in accordance with National Institute of Health Guidelines.

RESULTS AND DISCUSSION

For the preparation of the *in situ* forming drug delivery systems (*in situ* implants and ISM) PLGA was dissolved in 2-pyrrolidone. The 2-pyrrolidone was used throughout the

studies because it was found to have a better compatibility with skeletal muscle compared to NMP or DMSO (20). The degradation of the amorphous PLGA (RG 503) used in this study was expected within 6 weeks (25). Bupivacaine hydrochloride was chosen as a candidate for the development of a long-acting formulation for the treatment of acute and chronic pain. In order to maintain sink conditions the *in vitro* drug release was investigated in 0.01 M phosphate buffer pH 4.0 (bupivacaine hydrochloride solubility at pH 4.0, 37.53 mg/ml).

Upon injection of the polymer solutions (40% PLGA in 2-pyrrolidone) into the phosphate buffer medium, the polymer solidified as the solvent dissipated into the aqueous medium and formed implants as shown by scanning electron microscopy (Fig. 1a). Injection of the ISM system (polymer phase: 40% PLGA in 2-pyrrolidone; polymer/oil phase ratio 0.25:1) into the phosphate buffer medium led to the formation of microparticles (Fig. 1b). The *in situ* implant as well as the *in situ* microparticles were collected 24 h after injection into the buffer medium. In comparison to the *in situ* implants the particle surfaces of the *in situ* microparticles were less porous and more homogeneous.

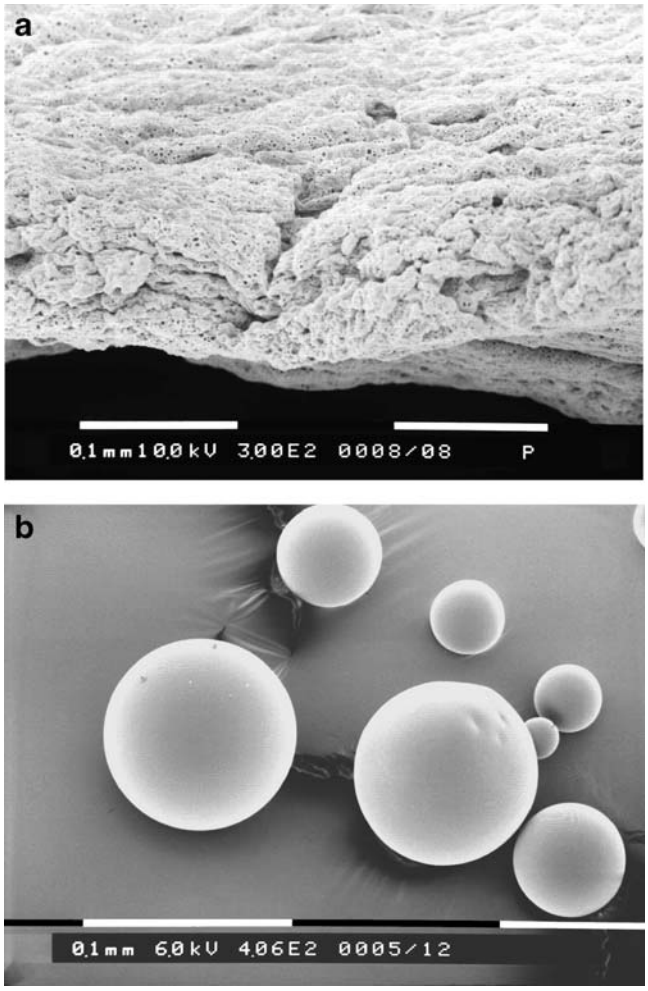

Fig. 1. Scanning electron micrographs of **a** a polymer solution (*in situ* implant, 40% PLGA in 2-pyrrolidone) and **b** an ISM system (polymer/oil phase ratio of 0.25:1, 40% PLGA in 2-pyrrolidone) upon injection into phosphate buffer medium.

In order to develop a suitable *in vitro* drug release system, the drug release from ISM formulations (polymer phase: 40% PLGA in 2-pyrrolidone; polymer/oil phase ratio 0.25:1) was investigated after placing the ISM emulsions into dialysis bags, one-site opened conical vials or after direct injection into the release medium (Fig. 2). The initial drug release decreased with the direct injection method > dialysis bags > vial method due to the increased contact surface between the aqueous buffer medium and the ISM emulsions. An optimal *in vitro* method needs to control variables such as sink conditions, reproducibility of sampling and the thickness of the diffusion layer. In addition, this method should predict *in vivo* release. Nevertheless, no optimal method has been developed yet. Since chemical hydrolysis of fatty oils was reported to proceed very slowly at physiological pH, an oily vehicle has to be cleared from the muscle in the solubilised state or by enzymatic degradation at the injection site (26). The muscular disappearance rate $t_{1/2}$ of peanut oil is 23 days (26). In order to mimic physiological conditions the ISM emulsions were placed into the dialysis bags or conical vials keeping the precipitated microparticles surrounded by a lipophilic environment. In contrast to the conical vials, drug diffusion from the dialysis bags was not restricted to one-site, which is comparable to the physiological environment and explains the faster drug release from dialysis bags compared to the conical vials. Furthermore, the handling at each sampling point was much easier using the dialysis bags compared to the direct injection method. Therefore, the dialysis membrane method was used for further *in vitro* release experiments.

ISM formulations can be developed either as a ready to inject system using the classical emulsification techniques such as Ultra-Turrax mixing and sonication or by emulsion formation just prior to injection by using two syringes, which are connected to each other by a polypropylene connector. The polypropylene connector had an inner diameter of 1.5 mm and is already commercially used for suspending microparticles into the suspension vehicle prior to injection into humans. The influence of the different preparation parameters on the *in vitro* drug release was investigated on ISM systems containing 40% PLGA in 2-pyrrolidone and a polymer/oil phase ratio of 0.25:1 (Fig. 3). After 48 h the drug release from the two-syringe system (34.1%) was slightly slower compared to the ready to inject systems (37.7 and 39.0% for the formulations prepared by Ultra-Turrax mixing or sonication, respectively). The particle size of the ISM decreased in the rank order of two-syringes system > sonication = Ultra Turrax mixing (Fig. 4). According to the Noyes–Whitney relation:

$$\frac{dM}{dt} = \frac{A\ D(c_s - c_t)}{h}$$

where dM/dt is the dissolution rate, A the specific surface area, D the diffusion coefficient, h the diffusion layer thickness, c_s the saturation solubility, and c_t the instantaneous drug concentration the drug release increases with increasing surface areas. Therefore, the rank order of particle sizes (two-syringes system > sonication = Ultra Turrax mixing) is in good agreement to the rank order of drug release (two-syringes system < sonication = Ultra Turrax mixing).

Bupivacaine hydrochloride containing *in situ* implants (40% PLGA solution in 2-pyrrolidone, 1:0 denoted in the

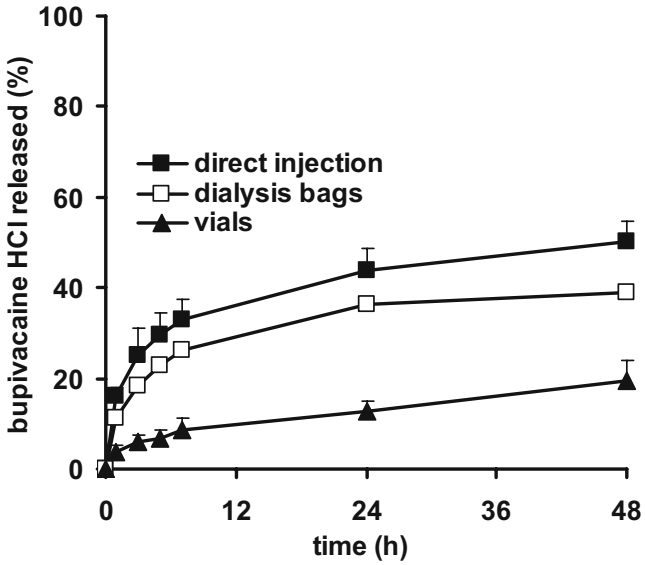

Fig. 2. Evaluation of different release methods for their potential use for the *in vitro* drug release studies from ISM formulations (polymer/oil phase ratio of 0.25:1, 40% PLGA in 2-pyrrolidone).

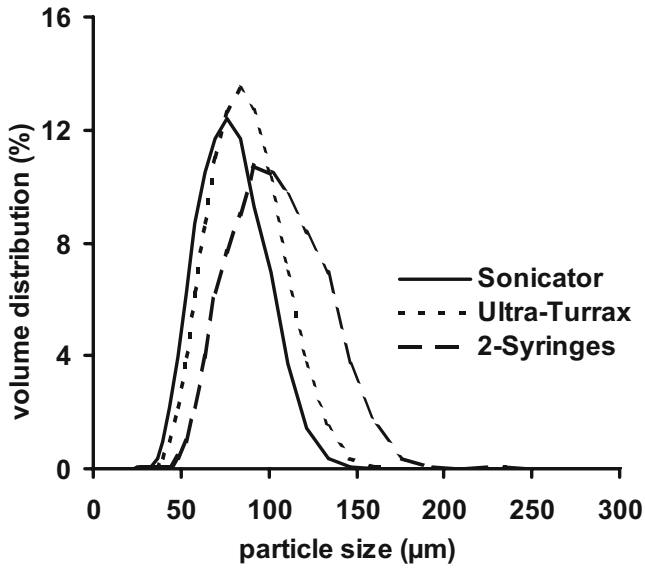

Fig. 4. Effect of different dispersion methods on the particle size distribution of ISM formulations (polymer/oil phase ratio of 0.25:1, 40% PLGA in 2-pyrrolidone) upon contact to phosphate buffer.

figure) showed a high initial drug release with 79.4% bupivacaine hydrochloride being released within 48 h (Fig. 5). With the ISM systems (polymer phase 40% PLGA in 2-pyrrolidone), the initial drug release decreased with decreasing polymer/oil phase ratio. After 48 h, 39.0 and 29.9% of the bupivacaine hydrochloride were released from ISM systems with a polymer/oil phase ratio of 0.25:1 and 0.1:1, respectively. The high initial drug release from *in situ* forming implants based on water miscible solvents is in good agreement with the literature (14). Due to the network of interconnecting pores, the active diffuses rapidly into the aqueous medium. This morphology has been described typically for a rapid phase inversion system. A reduced initial burst effect has been described when the aqueous affinity of the depot solvents was reduced (14). In contrast to the *in situ* implants the polymer phase of the ISM systems was emulsified into an oil, which formed a partial barrier (increasing with decreasing polymer/oil phase ratio) between the aqueous medium and the internal polymer solution. The low solubility of the active agent in the external oil phase caused the drug to stay in the inner polymer phase as it was encapsulated within the precipitated microparticles. In addition, the ISM had a less porous particle surface compared to the *in situ* implants (Fig. 1), this being a further possible explanation for the reduced initial drug release from ISM.

In order to study the ability of the ISM systems as an alternative for parenteral drug delivery, bupivacaine hydrochloride release from ISM systems (40% PLGA in 2-pyrrolidone, polymer/oil phase ratio of 0.1:1) was compared

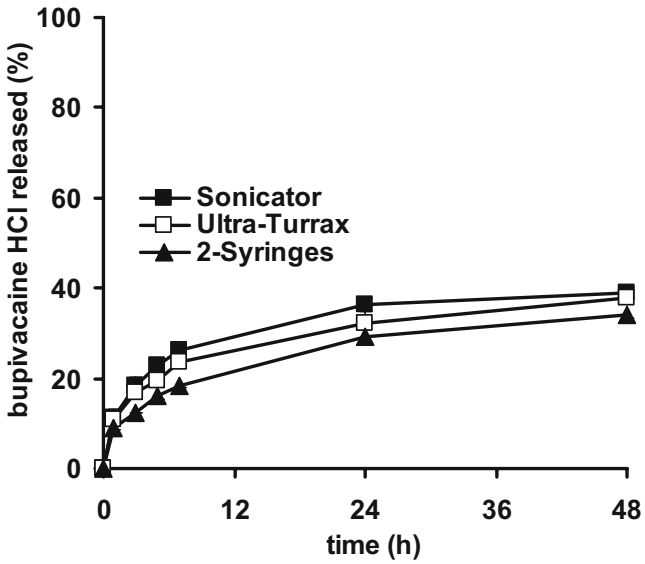

Fig. 3. Effect of different dispersion methods on the *in vitro* drug release from ISM formulations (polymer/oil phase ratio of 0.25:1, 40% PLGA in 2-pyrrolidone).

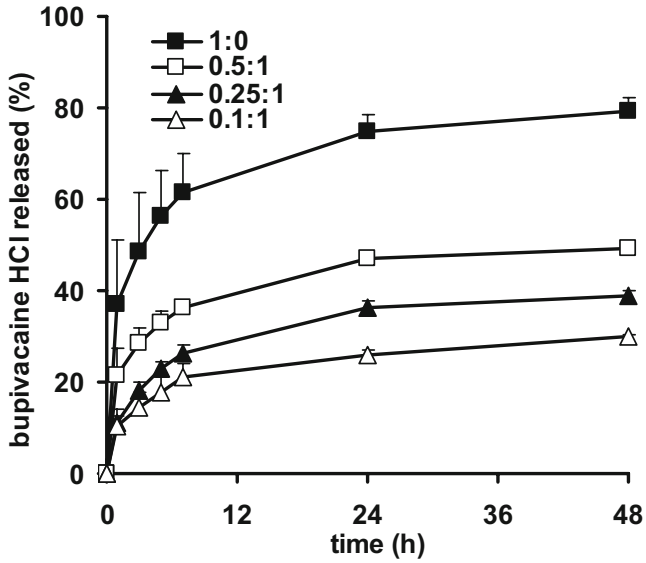

Fig. 5. Effect of the polymer phase/oil phase ratio on the drug release of ISM systems containing 40% PLGA solutions in 2-pyrrolidone as the inner polymer phase in comparison to a 40% PLGA solution in 2-pyrrolidone (1:0, *in situ* implant).

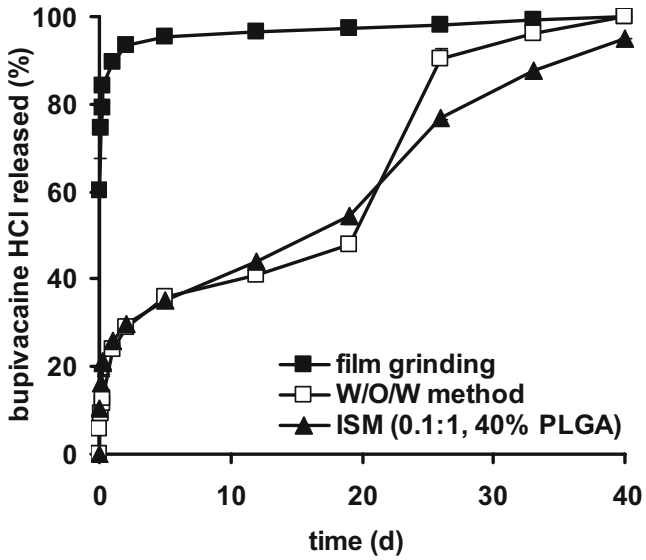

Fig. 6. Bupivacaine hydrochloride release from microparticles prepared by different conventional methods in comparison to the drug release from ISM (polymer/oil phase ratio of 0.1:1, 40% PLGA in 2-pyrrolidone) for up to 40 days.

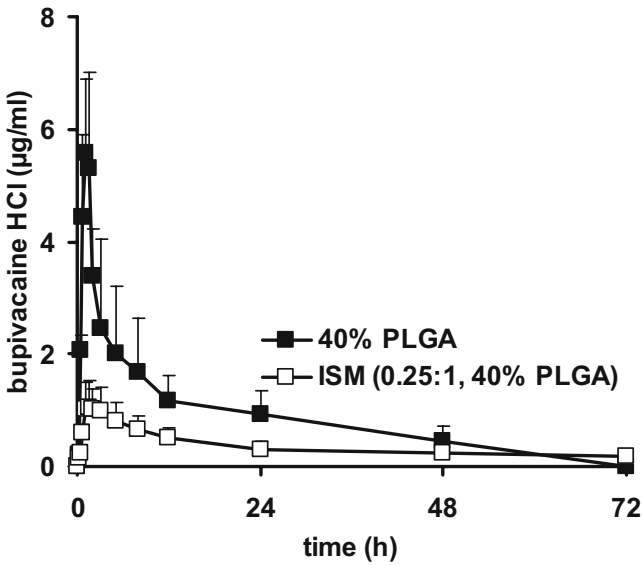

Fig. 7. Mean plasma bupivacaine hydrochloride levels versus time following the intramuscular injection of 5 mg drug using *in situ* implants (40% PLGA in 2-pyrrolidone) and ISM (polymer/oil phase ratio of 0.25:1, 40% PLGA in 2-pyrrolidone).

to microparticles prepared by film grinding or the W/O/W solvent evaporation method for up to 40 days (Fig. 6). The bupivacaine hydrochloride release from the microparticles prepared by the solvent evaporation method and the *in situ* technology was triphasic. After an initial burst, with approximately 25% drug being released after 1 day, the drug release rate was slower for the following 20 days. After 20 days, the rate of drug release increased significantly. Drug release from microparticles has been described to occur by diffusion through water-filled pores and after hydrolytic degradation/erosion of the polymer (25,27). The degradation of PLGA occurs in two steps, first chain cleavage as indicated by a decreasing molecular weight and second polymer erosion as indicated by a loss of matrix weight. Depending on the molecular weight of PLGA there is an induction period prior to mass loss or polymer erosion. High molecular weight polymers degrade to lower molecular weight fractions, which still remain water-insoluble. At a critical molecular weight of approximately 15,000 Da also water soluble fragments are formed and polymer erosion starts (27). The onset of polymer erosion of the PLGA (M_w 42,800) used in this study occurs after approximately 20 days (25). The degradation process of the polymer explains the triphasic release pattern with an initial drug release prior to polymer hardening (ISM) or due to non-encapsulated drug (microparticles), the lag time due to insufficient polymer degradation and increased drug release due to polymer erosion. These results also indicate that similar release profiles can be obtained when using the *in situ* microparticle technology instead of the complex solvent evaporation method, where many process and formulation parameters have to be controlled. In contrast, after one day almost all bupivacaine hydrochloride was released from microparticles prepared by film grinding. The high initial drug release from microparticles prepared by film grinding is in good agreement to other studies (28). This can be explained with the highly accessible surfaces after grinding. Microparticles prepared with other methods usually have a skin on the surface, which acts as a diffusion barrier. Oily drug suspensions have been reported to be a possible alternative for the prolonged delivery of lipophilic active agents. However, an oily drug suspension was not an alternative for the controlled delivery of bupivacaine hydrochloride. Almost all active agent was released from an oily drug suspension within 48 h. These findings are in good agreement to a previous study (29).

Based on the *in vitro* results, *in vivo* studies were performed on selected formulations. The systemic drug

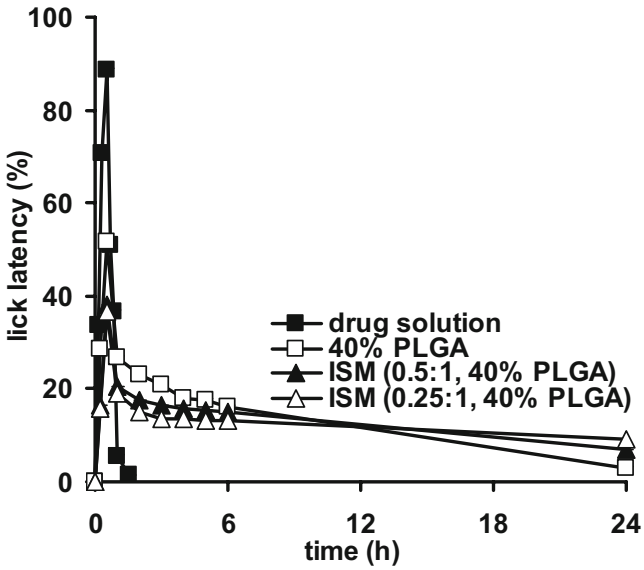

Fig. 8. Mean lick latency values versus time following the intramuscular injection of 5 mg bupivacaine hydrochloride in 2-pyrrolidone (drug solution), 40% PLGA in 2-pyrrolidone (*in situ* implant) or ISM (polymer to oil phase ratios of 0.5:1 and 0.25:1, 40% PLGA in 2-pyrrolidone).

plasma concentration was determined, following the intramuscular injection of a polymer solution or an ISM formulation (Fig. 7). ISM with a polymer/oil phase ratio of 0.25:1 were chosen for *in vivo* studies as these systems provided a significant reduced initial drug release *in vitro*. The administered bupivacaine hydrochloride dose was 5 mg for the 40% PLGA solution in 2-pyrrolidone and the ISM system (40% PLGA in 2-pyrrolidone, polymer/oil phase 0.25:1). The c_{max} value for the polymer solution was 5.57 μg/ml and was reached after 1 h. The c_{max} of 1.06 μg/ml for the ISM system was reached after 1.5 h. These results indicate that drug-containing ISM formulations resulted in a reduced initial drug release, thus decreasing the systemic uptake of the local anesthetic compared to the drug containing polymer solution. Plasma concentrations above 2.0 μg/ml have been reported to be the threshold for central nervous system toxicity in humans or rats (3). Therefore, the ISM can lead to a reduction of the severity of bupivacaine induced systemic side effects, when compared to the polymer solution. Using the same dose of bupivacaine peak plasma concentrations following the injection of the ISM into rats were approximately 10 times higher than absolute plasma values following the injection of microparticle prepared by the solvent evaporation method into rabbits (9). This can be explained with the higher distribution volume within the rabbits that leads to lower peak plasma levels. Nevertheless, the shape of the apparent elimination phase of the plasma concentration versus time profile from ISM injected into rats was similar to the elimination profile resulting from the injection of microparticles prepared by the solvent evaporation method into rabbits (9), suggesting a sustained release of the drug.

Several animal models have been developed to evaluate the physiologic and pharmacologic effects induced by the administration of local anesthetics and opioids (30). Besides measurements of the intensity of motor block by investigating the walking disturbances of rats or rabbits (6,10), a hot plate model has been used for the pharmacodynamic quantification of sustained local anesthetic effect (3,8). Following the implantation of bupivacaine loaded polyanhydride implants (8) or the injection of bupivacaine loaded PLGA microparticles (3) the sensory block of the rats was determined by assessing the lick latency.

A hot plate model has been used in order to quantify the pharmacodynamic effect of the bupivacaine release from *in situ* implants and microparticles in male Sprague–Dawley rats. Based on the reduced initial *in vitro* drug release ISM with a polymer/oil phase ratio of 0.25:1 and 0.5:1 were selected for *in vivo* studies. Control groups that were injected with either normal saline or 2-pyrrolidone alone showed no sensory blockade. Sensory blocks following the injection of 1 and 3 mg of bupivacaine hydrochloride in normal saline, polymer solution or ISM were less pronounced when compared to the effects of the 5 mg drug dose (data not shown). The maximum change in the lick latency (88.6%) occurred following the injection of the drug solution (5 mg bupivacaine HCL in normal saline) and was reached after 30 min (Fig. 8). The duration of the sensory block produced by the drug solution leveled off after 90 min, indicating the rapid elimination of the drug from the site of administration. A maximum change of 51.8, 38.0 and 36.8% for the polymer solution and the ISM at a polymer/oil phase ratio of 0.5:1 and 0.25:1 was reached after 30 min, respectively. The duration of the sensory block produced by the polymer solution and the ISM was prolonged compared to the drug solution. With the polymer solution, the effect on the lick latency leveled off after 6 h. A comparison between the lick latency of the control group which was not treated and of the ISM treated rats indicated a significant change of the lick latency for up to at least 24 h. Further, studies indicated an effect on the sensory block for up to 48 and 72 h for the rats treated with ISM at a polymer/oil phase ratio of 0.5:1 and 0.25:1, respectively. After 48 h the lick latencies for rats treated with ISM at a polymer/oil phase ratio of 0.5:1 and 0.25:1 were 5.0 and 10.0%. After 72 h the lick latencies decreased to 2.5 and 5.6% for rats treated with ISM at a polymer/oil phase ratio of 0.5:1 and 0.25:1. However, this effect needs to be verified at higher drug doses. Following the injection of a five times higher bupivacaine dose incorporated into microparticles produced by the solvent evaporation method a prolonged sensory blockade was observed for up to 5.5 days (3). For the ISM, injection of higher bupivacaine doses are possible as indicated by the low peak plasma levels.

In conclusion, the *in vitro* drug release from *in situ* forming drug formulations was highly influenced by the polymer to oil phase ratio. One advantage of the ISM system when compared to *in situ* implant systems (polymer solutions) was the significantly reduced burst effect due to the presence of an external oil phase. The *in vivo* drug release studies were in good agreement with the *in vitro* drug release. With the ISM system the pharmacodynamic effect of the bupivacaine HCL was prolonged compared to the injection of a drug solution or drug-polymer solution (*in situ* implant). Therefore, as an option to the multiple step preparation of conventional microparticles these simple drug carrier formulations in liquid form (ISM) are an attractive alternative for long-acting parenteral drug delivery systems.

REFERENCES

1. D. B. Masters, C. B. Berde, S. K. Dutta, C. T. Griggs, D. Hu, W. Kupsky, and R. Langer. Prolonged regional nerve blockade by controlled release of local anesthetic from a biodegradable polymer matrix. *Anesthesiology* **79**:340–346 (1993).
2. M. J. Cousins and P. O. Bridenbaugh. *Neural blockade in clinical anesthesia and management of pain*. Lippincott Williams & Wilkins, Philadelphia, 1988, pp. 719–735.
3. J. Curley, J. Castillo, J. Hotz, M. Uezono, S. Hernandez, J. O. Lim, J. Tigner, M. Chasin, R. Langer, and C. Berde. Prolonged regional nerve blockade. *Anesthesiology* **84**:1401–1410 (1996).
4. L. Bonhomme, D. Benhamou, and T. Beugre. Slow release effect of pH-adjusted bupivacaine: *in vitro* demonstration. *Int. J. Pharm.* **84**:33–37 (1992).
5. J. R. Mhando and A. Li Wan Po. Controlled release of local anaesthetic agents from liquid–solid emulsion gels. *Int. J. Pharm.* **62**:249–258 (1990).
6. L. Langerman, J. G. Gilbert, M. Zakowski, E. Golomb, S. Ramanathan, and H. Turndorf. Prolongation of epidural anesthesia using a lipid drug carrier with procaine, lidocaine, and tetracaine. *Anesth. Analg.* **75**:900–905 (1992).
7. A. Gesztes and M. Mezei. Topical anesthesia of the skin by liposome encapsulated tetracaine. *Anesth. Analg.* **67**:1079–1081 (1988).

8. D. B. Masters, C. B. Berde, S. Dutta, T. Turek, and R. Langer. Sustained local anesthetic release from bioerodible polymer matrices: a potential method for prolonged regional anesthesia. *Pharm. Res.* **10**:1527–1532 (1993).
9. J. P. Estebe, P. Le Corre, Y. Mallédant, F. Chevanne, and R. Leverge. Prolongation of spinal anesthesia with bupivacaine-loaded (D,L-lactide) microspheres. *Anesth. Analg.* **81**:99–103 (1995).
10. J. M. Malinovsky, J. M. Bernard, P. Le Corre, J. B. Dumand, J. Y. Lepage, R. Le Verge, and R. Souron. Motor and blood pressure effects of epidural sustained-release bupivacaine from polymer microspheres: a dose-response study in rabbits. *Anesth. Analg.* **81**:519–524 (1995).
11. R. Jalil and J. R. Nixon. Biodegradable poly(lactide acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *J. Microencapsulation* **7**:297–325 (1990).
12. R. L. Dunn, J. P. English, D. R. Cowsar, and D. P. Vanderbilt. Biodegradable *in situ* forming implants and methods of producing the same. US Patent 4,938,763 (1990).
13. N. H. Shah, A. S. Railkar, F. C. Chen, R. Tarantino, S. Kumar, M. Murjani, D. Palmer, M. H. Infeld, and A. W. Malick. A biodegradable injectable implant for delivering micro and macromolecules using poly(lactic-co-glycolic) acid (PLGA) copolymers. *J. Control. Release* **27**:139–147 (1993).
14. P. D. Graham, K. J. Brodbeck, and A. J. McHugh. Phase inversion dynamics of PLGA solutions related to drug delivery. *J. Control. Release* **58**:233–245 (1999).
15. E. G. Duysen, G. L. Yewey, J. L. Southard, R. L. Dunn, and W. Huffer. Release of bioactive growth factors from the Atrigel delivery system in tibial defect and dermal wound models. *Pharm. Res.* **10**:83 (1993).
16. A. Hatefi, and B. Amsden. Biodegradable injectable *in situ* forming drug delivery systems. *J. Control. Release* **80**:9–28 (2002).
17. C. B. Packhaeuser, J. Schnieders, C. G. Oster, and T. Kissel. *In situ* forming parenteral drug delivery systems: an overview. *Eur. J. Pharm. Biopharm.* **58**:445–455 (2004).
18. D. Steinberg and M. Friedman. Dental drug delivery devices: local and sustained release applications. *Crit. Rev. Therapeutic Drug Carrier Systems* **16**:425–459 (1999).
19. H. Kranz and R. Bodmeier. A biodegradable *in situ* forming system for controlled drug release. *PharmSci. Supplement* **1**:414 (1998).
20. H. Kranz, G. A. Brazeau, J. Napaporn, R. L. Martin, W. Millard, and R. Bodmeier. Myotoxicity studies of injectable biodegradable *in situ* forming drug delivery systems. *Int. J. Pharm.* **212**:11–18 (2001).
21. R. Bodmeier. Multiphase system. Int. Patent Application WO 98/55100 (1998).
22. W. J. Millard, J. J. Martin, J. Audet, S. M. Sagar, and J. B. Martin. Evidence that reduced growth hormone secretion observed in monosodium glutamate-treated rats is due to a deficiency in growth hormone-releasing factor. *Endocrinology* **110**:540 (1982).
23. P. Le Corre, P. Le Guevello, V. Gajan, F. Chevanne, and R. Le Verge. Preparation and characterization of bupivacaine loaded polylactide and poly-co-glycolide microspheres. *Int. J. Pharm.* **107**:41–49 (1994).
24. X. J. Xu, Z. Wiesenfeld-Hallin, J. Hughes, D. C. Horwell, and T. Hökfelt. CI 1988, a selective antagonist of cholecystokinin$_B$ receptors, prevents morphine tolerance in the rat. *J. Pharmacol.* **105**:591–596 (1992).
25. D. H. Lewis. Controlled release of bioactive agents from lactide/glycolide polymers. In M. Chasin and R. Langer (eds.), *Biodegradable Polymers as Drug Delivery Systems*, Dekker, New York, 1990, pp. 1–41.
26. K. Schultz, B. Mollgaard, A. N. Fisher, L. Illum, and C. Larsen. Intramuscular rate of disappearance of oily vehicles in rabbits investigated by gamma-scintigraphy. *Int. J. Pharm.* **169**:121–126 (1998).
27. A. Rothen-Weinhold, K. Besseghir, Y. De Zelicourt, and R. Gurny. Development and evaluation *in vivo* of a long-term delivery system for vapreotide, a somatostatin analogue. *J. Control. Release* **52**:205–213 (1998).
28. K. El-Kharraz, A. Dashevsky, and R. Bodmeier. Microparticles prepared by grinding of films. Proceedings of the 2nd World Meeting APGI/APV, Paris, pp. 555–556 (1998).
29. H. Kranz and R. Bodmeier. A novel *in situ* forming drug delivery system for controlled parenteral drug delivery. *Int. J. Pharm.* **332**:107–114 (2007).
30. L. Langerman, G. Chaimsky, E. Golomb, M. Tverskoy, A. Kook, and S. Benita. A rabbit model for evaluation of spinal anesthesia: chronic cannulation of the subarachnoid space. *Anesth. Analg.* **71**:529–535 (1990).