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PREPARATION AND EVALUATION OF METHOTREXATE-LOADED BIODEGRADABLE POLYANHYDRIDE MICROSPHERES

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ABSTRACT

Methotrexate-loaded biodegradable polyanhydride microspheres were prepared by modified hot-melt technique and aqueous solvent evaporation technique. The effect of particle size, drug loading and microencapsulation technique on the in vitro drug release was studied. The in vitro release of methotrexate was evaluated using an automated flow-through cell system. The release profile consisted of burst release and sustained release phases. The burst release from the microspheres prepared by the modified technique was lower than that from the aqueous solvent evaporation technique. In addition, the microspheres with lower loadings released smaller amounts during the burst release phase. For a given loading and processing technique, the amount released by burst decreased with an increase in particle size. The microspheres prepared by the modified hot-melt technique with 10% loading and 177-250 μm size fraction gave desirable prolonged release. This formulation was tested in vivo in rats by subcutaneous implantation. The peak serum level of

methotrexate was reached between 15-18 hours compared to that between 0-3 hours observed following the administration of an equivalent dose of methotrexate solution. No microspheres were found at the site of implantation at 48 hours post-implantation.

INTRODUCTION

Methotrexate (MTX) is a chemotherapeutic agent used in the treatment of several forms of cancer, including acute lymphoblastic leukemia in children (1,2). It is administered in high doses either as an infusion or orally. The administration of large amounts of MTX by the conventional routes results in systemic toxicity (1,3). In addition, the bioavailability of its oral administration is unpredictable. In order to ensure complete bioavailability and avoid the inconvenience and expense of continuous infusion of long duration, administration of MTX in a biodegradable implantable drug delivery system was evaluated. Such a system would have the added benefit of providing methotrexate at the site of tumor and hence, may result in reduced systemic dose and therefore, reduced toxicity. This approach has been used for several applications that require localized treatment such as dental implants for dental caries and gum diseases and, wound dressings (4).

For ease of administration, microsphere formulations were preferred over implants. Polyanhydrides are a class of biodegradable polymers that have been used in preparation of prolonged delivery systems for many conventional drugs as well as protein molecules (5,6). Several techniques for preparation of polyanhydride microspheres have been reported, including solvent evaporation, solvent extraction and hot-melt techniques (7,8). The use of solvent evaporation technique may result in loss of water-soluble active drug and loss in the molecular weight (MW) of the polymer due to hydrolysis. The solvent extraction and hot-melt techniques produce spherical particles with low molecular weight polyanhydrides (MW < 20,000); with higher molecular weight polyanhydrides, either lump or rod shaped particles are obtained (6).

Therefore, MTX-loaded polyanhydride microspheres were prepared with Biodel® (a copolymer of p-bis-carboxyphenoxy propane and sebacic acid in 1:4 weight ratio; abbreviated as p(CPP:SA) 20:80) for 24-36 hour release of the drug. A modified hot-melt (combination of hot-melt & solvent extraction) technique was developed for preparation of microspheres of high molecular weight Biodel® (MW > 20,000) containing MTX, a water soluble drug.

MATERIALS AND METHODS

Materials

Methotrexate, NF (Farmos Group, Ltd.) and Biodel® (MW 50,000) were provided by Nova Pharmaceutical Corp., Crodesta F-110 was donated by Croda, Inc., dibasic and monobasic sodium phosphate, methylene chloride, ethyl ether and paraffin oil were obtained from VWR Scientific Co., Span 85 was donated by ICI America, Inc. and carboxymethyl cellulose was donated by Aqualon Co. MTX was dried prior to use as described below. All other materials were used as received.

Moisture Content of MTX

The moisture content of the MTX received was determined using a Fisher Automatic K-F titrimeter system. MTX was then vacuum dried under a pressure of 1 μm of mercury and a temperature of 140°F for 16 hours. The moisture content was determined at the end of the drying cycle.

Preparation of Microspheres

Microspheres of polyanhydride were prepared using two different techniques namely, solvent evaporation and modified hot-melt technique.

For preparation of microspheres by solvent evaporation technique, 5 g of Biodel® was dissolved in 20 g of methylene chloride. To this, 500 mg of MTX was added and dispersed uniformly using a sonicator probe

(Branson Ultrasonic, model LS75) at power level 3 for 5 seconds. Separately, 150 ml of 1.0% w/v Crodesta F-110 aqueous solution was prepared in a 400 ml capacity tall glass beaker equipped with an impeller stirrer. The MTX dispersion prepared was added to the surfactant solution under agitation. The mixing was continued for another 3 hours and methylene chloride was allowed to evaporate until the microspheres were obtained. The microsphere suspension was filtered through standard US sieves to obtain desired particle size fractions. The microspheres were collected and washed with distilled water. The size separated microspheres were then vacuum dried for 12 hours and stored at -15 to -20°C until further evaluation. Different MTX loading levels were obtained by varying the ratio of Biodel® to MTX. The microspheres were prepared by solvent evaporation technique with initial MTX loadings of 10, 20 and 40% and particle size ranges from 149 to 500 μm.

For preparation of microspheres using modified hot-melt technique, MTX dispersion in Biodel® solution was prepared as described above. Separately, 100 g of heavy mineral oil containing 2.0% w/w of span 85 was heated to 75-80°C in a jacketed glass beaker equipped with an impeller stirrer. With vigorous mixing, the MTX dispersion was added to paraffin oil and the mixing was continued for further 60 seconds. The stirring speed was reduced and ice-chilled water was circulated through the outer jacket of the beaker to quickly cool the emulsion to 10-15°C and solidify the microspheres. The microsphere dispersion was then filtered through US sieve # 150 and rinsed with ethyl ether. In order to remove any adhering mineral oil, the microspheres were kept overnight in ether. The microspheres were then size separated through standard US sieves and washed with ethyl ether. The size separated microspheres were vacuum dried and stored as described earlier. Modified hot-melt technique was used to prepare microspheres at 10 and 20% loading and particle size ranges from 149 to 250 μm.

In Vitro Evaluation of Microspheres

The microspheres were evaluated for appearance, surface morphology, and MTX release. The appearance of the microspheres was

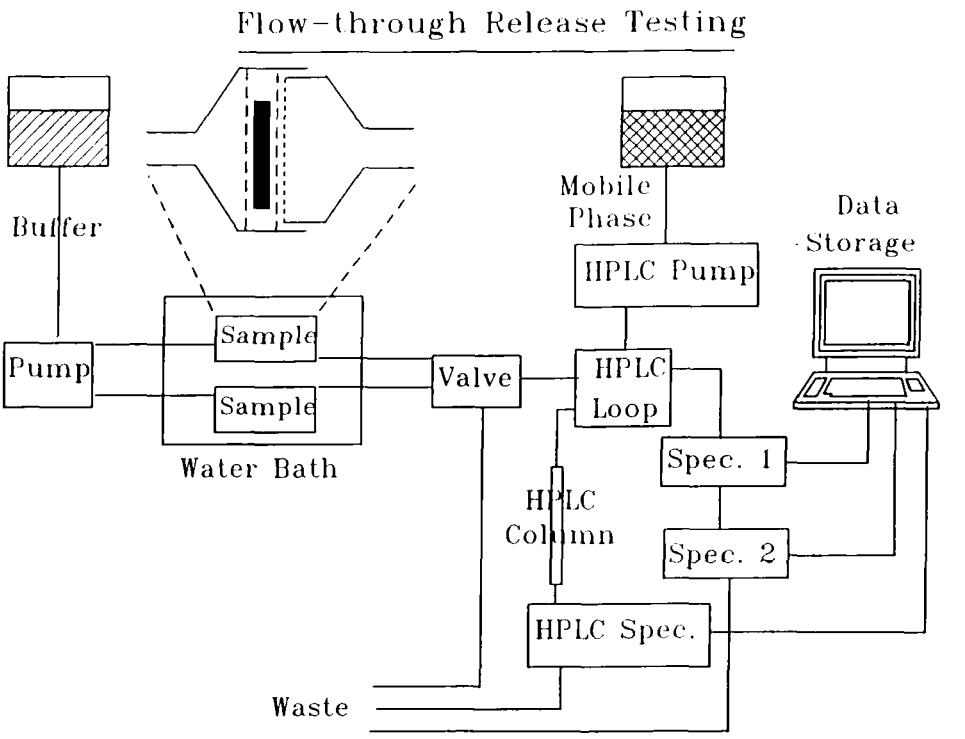

FIGURE 1
Schematic representation of automated flow-through cell system (sample enlarged into the filter holder with glass wool).

noted visually and under a stereo optical microscope (Olympus Corp., model SZH). The surface morphology of the microspheres was evaluated using a scanning electron microscope (SEM). The dried microspheres were sputter coated by gold/palladium and then observed under the SEM.

In vitro release of MTX from the microspheres was evaluated using a modified automated flow-through cell system (9) (Fig. 1). About 20-40 mg of microspheres were sandwiched between two glass wool filters and placed in a filter holder. The flow-cell device thus prepared was placed in a water bath maintained at $37\pm1^{o}C$. The inlet end of the flow-cell was connected to a peristaltic pump delivering isotonic phosphate buffer, pH 7.4 at 0.6 ml/minute. The outlet end of the flow-cell was connected to a solenoid valve which directed the flow either to a spectrophotometer

through a HPLC loop or, to a waste container. The solenoid valve was controlled by a computer which also collected data from the HPLC detector and the spectrophotometer. Upto four flow-cells were used at a time and the computer was programmed such that the effluent from each cell was monitored for 15 minutes every hour. MTX concentration in the effluent was determined by using a modified compendial HPLC assay procedure for MTX.

In Vivo Evaluation of Microspheres

Male Sprague-Dawley rats (248-307 gm), housed in cages kept in a Bio-clean room with unrestricted access to both food and water were used in this study. Sixteen rats were implanted with the microspheres prepared by the modified hot melt technique at 10% MTX loading and 177-250 μm size fraction with a dose equivalent to 5 mg/kg of MTX. The microspheres were suspended in 0.1% carboxymethyl cellulose in sterile saline and implanted subcutaneously into the nape of the neck of the anaesthetized animal by an incision. A second group of animals were administered 5 mg/kg equivalent of methotrexate powder dissolved in the vehicle and four rats received the vehicle alone. At 3, 6, 9, 12, 15, 18, 24 and 48 hours, two animals from the first group and one animal from the second group were sacrificed and blood samples were collected by cardiac puncture. Serum from the samples was analyzed for MTX concentration by an automated fluorescence immunopolarization analyzer, the Abbott TDx. The working range of the assay was found to be 0.01- 1.0 μM/L, with mean recovery of MTX spiked into serum of 97.6±2.8%. The data from reproducibility studies using replicates (n=5) of spiked human serum yielded coefficients of variation of less than 10%. The only compounds that demonstrated a significant (>1%) cross reactivity when tested with the TDx MTX assay, had a similar chemical structure (n=2), and were present at relatively high concentrations (6-100 μM/L) with MTX. The site of implantation was removed post-mortem and examined for any remaining microspheres.

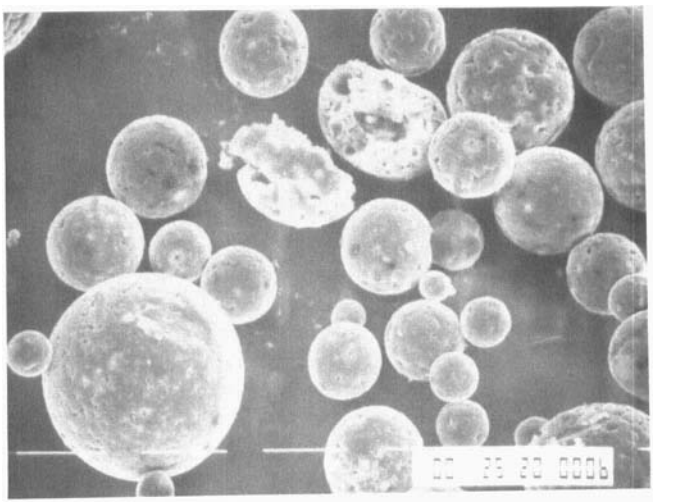

FIGURE 2
Scanning electron micrograph of microspheres prepared by modified hot melt technique (magnification 150X).

RESULTS AND DISCUSSION

The MTX received was found to contain 10% moisture. Prior to microencapsulation, it was necessary to eliminate this moisture in order to minimize hydrolysis of the biodegradable polyanhydride during the microencapsulation process using the modified hot-melt technique. The drying of MTX lowered the moisture content to 2%.

The modified hot-melt technique resulted in successful microsphere formation. This was evident from the scanning electron micrographs (Fig. 2) which revealed spherical shape with a porous surface and interior. The microspheres prepared by the solvent evaporation technique (Fig. 3) had a continuous but uneven surface. The total drug content of microspheres prepared using modified hot-melt

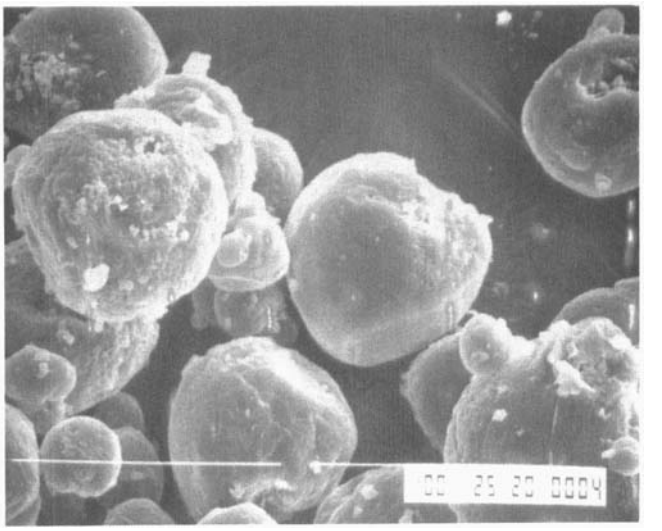

FIGURE 3
Scanning electron micrograph of microspheres prepared by solvent evaporation technique (magnification 500X).

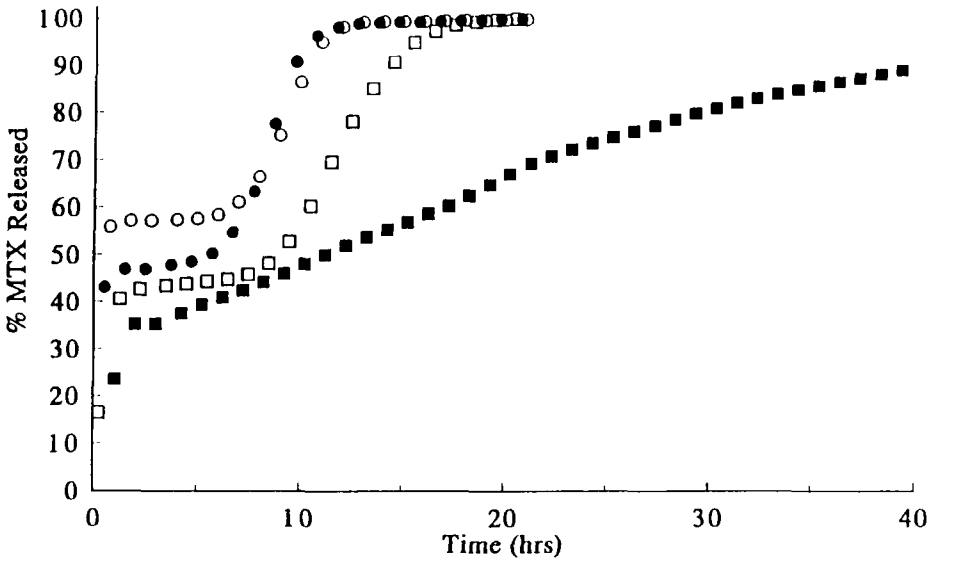

FIGURE 4
MTX release from microspheres prepared by solvent evaporation technique: 10% loading, 149-177 μm (○), 177-250 μm (●), 250-420 μm (□) and 420-500 μm (■).

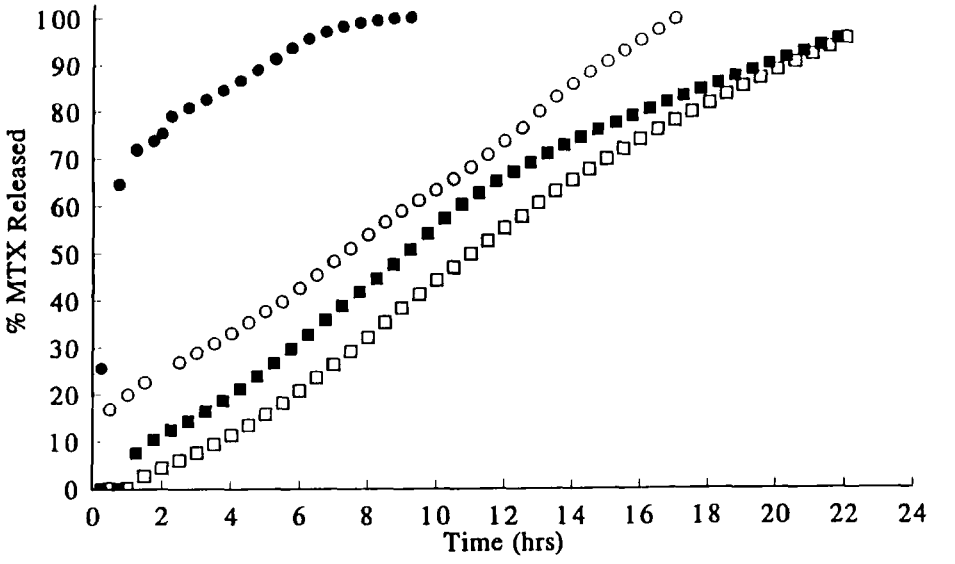

FIGURE 5
MTX release from microspheres prepared by modified hot melt technique: 10% loading, 149-177 μm (■) and 177-250 μm (□); 20% loading, 149-177 μm (●) and 177-250 μm (○).

technique was close to 100% of the theoretical content. However, microspheres prepared using solvent evaporation technique lost a significant amount of drug to the aqueous continuous phase (20% of theoretical content at 10% initial drug loading).

The release profile of MTX from the microspheres prepared by solvent evaporation technique is depicted in Figure 4. It was characterized by an initial burst due to the surface MTX, followed by a slow release due to diffusion. The second burst occurred due to degradation of the polymer by bulk erosion and then a slow release again by diffusion. The duration of MTX release decreased with a decrease in particle size due to faster diffusion and erosion (Fig. 4) and an increase in initial MTX concentration (data not shown). The microspheres prepared by the modified hot-melt technique provided a smaller initial burst of MTX at the 10% drug loading (Fig. 5) and a longer duration of release. The

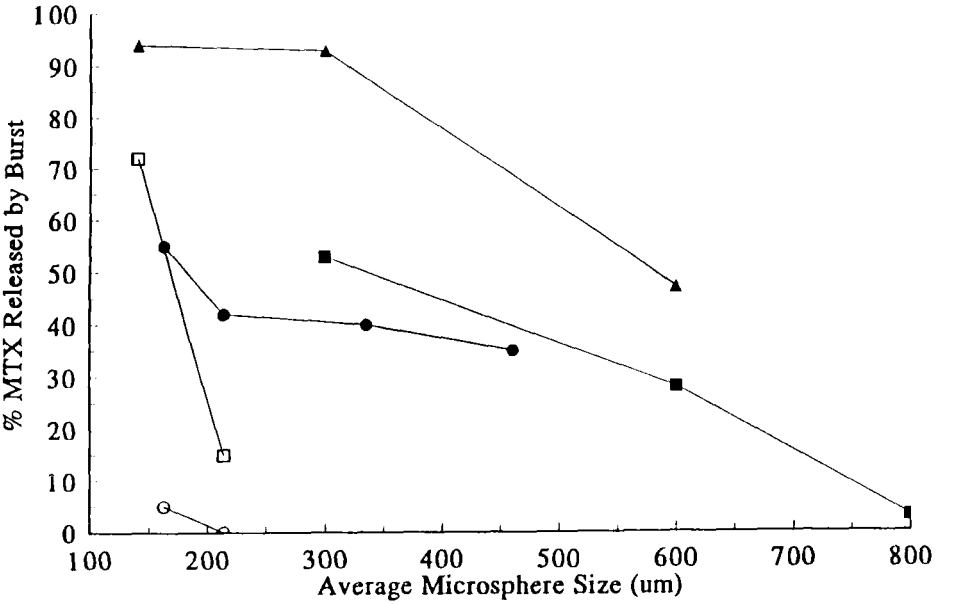

FIGURE 6

Effect of loading, microsphere size and processing technique on the burst release from the microspheres: solvent evaporation technique, loadings of 10% (●), 20% (■) and 40% (▲) modified hot-melt technique, loadings of 10% (○) and 20% (□).

effect of MTX loading, microsphere size and microencapsulation procedure on initial burst obtained is shown in Fig. 6. Greater amount of MTX burst is seen from the microspheres prepared by the solvent evaporation technique than from those prepared by the modified hot-melt technique. However, the initial burst from the 20% MTX-loaded microspheres prepared by the hot-melt technique is higher than that from 10% MTX-loaded microspheres prepared by the solvent evaporation method. This indicates a limit on the loading for efficient encapsulation of MTX for controlled release. For a given technique, greater burst of the drug is seen with increase in the drug loading. As the microsphere particle size increases, slower bulk erosion of the microspheres decreases the amount of burst release. The microspheres prepared using the modified hot-melt technique with 10% MTX loading and 177-250

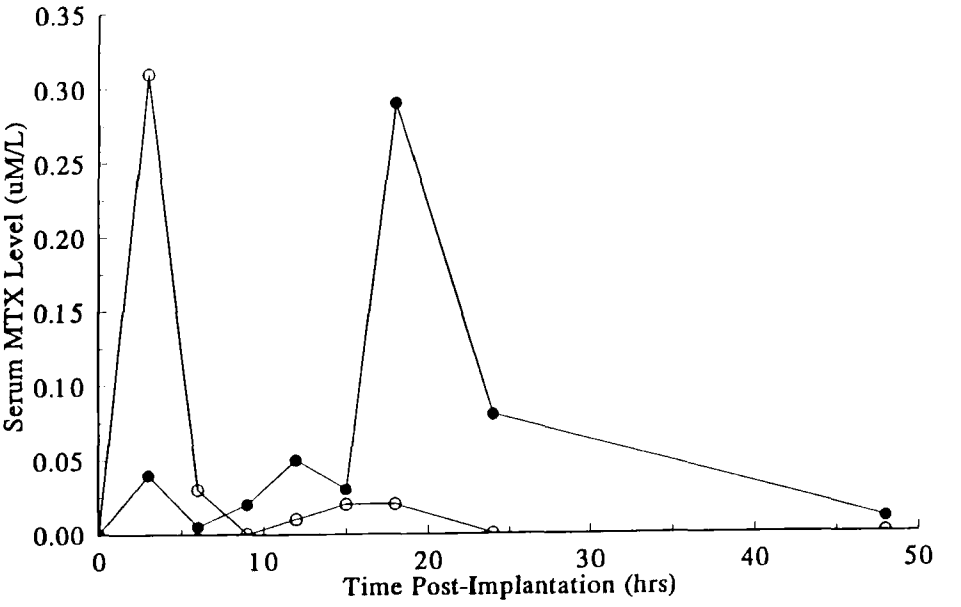

FIGURE 7
Serum MTX concentration-time profile: MTX solution (O) and MTX-Biodel® microspheres (●), 10% loading and 177-250 μm size fraction.

μm size fraction provided acceptable in vitro release profiles and were used for in vivo evaluation.

In vivo, T_{max} for the microspheres formulation occurred between 15-18 hours post-implantation, giving a mean C_{max} of 0.29 μM/L (n=2). Very little MTX was measured at 24 hours, and no MTX was detectable at the 48 hour sampling time (Fig. 7). The T_{max} for the MTX solution occurred between 0-3 hours. The area under the curve (AUC) calculated from the serum concentration-time data of the MTX-loaded microspheres had a mean value of 3.02 μM.hr/L, while that for the SC implantation of the MTX solution was 1.2 μM.hr/L. This may not accurately reflect the total amount of drug absorbed from MTX solution since frequent early time points were not available. Post-mortem observations of the implantation sites revealed a decrease in microsphere size at 24 hours post-implantation, with the microspheres disappearing almost completely at 48 hrs post-implantation.

CONCLUSIONS

The modified hot-melt technique developed yields spherical microspheres of high molecular weight polyanhydrides with minimal loss of drug during preparation. In vitro, MTX release from the microspheres occurs in phases, burst followed by prolonged release depending on the loading, size and method of preparation of the microspheres. Larger burst release was observed from the microspheres prepared by the solvent evaporation technique than from those prepared by the modified hot-melt technique. An increase in MTX loading and a decrease in microsphere size resulted in an increase in percent released by burst and a decrease in duration of release. In vivo, prolonged MTX release was obtained from the microspheres. These preliminary results show promise for controlled release and warrant further investigation.

ACKNOWLEDGMENTS

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