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International Journal of Pharmaceutics 122 (1995) 9–15

international journal of pharmaceutics

Method of evaluation of sustained release microsphere formulations using the open chemostat system

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Received 25 March 1994; revised 21 September 1994; accepted 29 December 1994

Abstract

The sustained release of cephalexin (CPX) as the monohydrate and ciprofloxacin (CFX) as the hydrochloride from separate 100000 Mol. Wt poly(L-lactic acid) microspheres (250–425 μm sieve size range) was evaluated in an open chemostat system. Drug concentrations in pH 7.4 phosphate buffer solution (PBS) reached peak levels which were sustained for different periods of time, depending on the flow rate of PBS at a constant volume of 120 ml in the chemostat. At 0.46 ml/min flow rate CPX microspheres (33% w/w loading) sustained CPX concentrations for approx. 90 min (solubility = 40 mg/ml at pH 7.4, 37° C) whereas CFX (solubility = 5 mg/ml) was sustained for at least 6 h. Increasing drug loading increased peak levels of either antibiotic but decreased the sustained period of CPX only. Decreasing microsphere size to 125–250 μm increased CPX or CFX concentration levels and decreased the sustained period to about 45 min and 2 h, respectively. At higher doses, CFX was sustained at higher concentrations over the 6 h period. Decreasing the flow rate in the chemostat increased sustained levels of CFX while increasing the flow rate decreased sustained levels. Thus, the chemostat system is convenient for testing the sustained release of drugs as a function of formulation parameters and to obtain information about the optimum doses of sustained release medication for in vivo administration.

Keywords: Open chemostat; Sustained release testing; Cephalexin; Ciprofloxacin; Poly(L-lactic acid) microsphere

1. Introduction

Various methodologies have been employed to determine the kinetics of release of therapeutic agents from sustained or controlled release formulations (Brossard et al., 1983; Nicklasson et al., 1983; Bodmeier and Chen, 1990; Fukumori et al., 1991). These include, for the most part, closed systems (e.g., USP methods) in which the accumulated amounts of drug released are measured. Although this may be satisfactory for comparisons of different formulations, the data cannot be extrapolated to represent drug concentrations in vivo because of the dynamics of simultaneous drug input and clearance at the site of absorption in a biological system, for example, after i.p. administration. Washington and Koosha (1990) have described a flow-through filtration cell for the study of release from sustained release microspheres. Also, an open, flow-through method that

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SSDI 0378-5173(95)00029-1

operates under sink conditions, exemplified by the Langenbucher system, has been used mainly for dissolution testing of tablets (Wennergren et al., 1989; Nicklasson et al., 1991) but has problems of filter clogging and pressure build-up (Abdou, 1990).

An open chemostat system which has been routinely used to study the kinetics of interaction between antibiotics and bacteria, allowing simple, convenient determination of the required dose for administration (Anwar et al., 1992) can be modified to study fluid concentrations of drug from sustained release polymeric dosage forms, such as microparticle formulations.

2. Materials and methods

Poly(L-lactic acid) (Mol Wt 100000) was obtained from Polysciences Inc. (Warrington, PA). Cephalexin monohydrate (CPX) and ciprofloxacin hydrochloride (CFX) were purchased from Sigma Chemical Co., St. Louis, Mo. Diethyl ether and all other chemicals were reagent grade. Demineralized, double-distilled water was used throughout the study.

2.1. Preparation of microspheres

Typically, a sieve-sized fraction of CFX or CPX powder less than 250 μm was suspended in 10 ml methylene chloride containing 400 mg PLA and stirred (Ikamag magnetic stir plate, 400 rpm). Diethyl ether (36 ml) as the nonsolvent was delivered to the stirred solution at a constant rate of 0.2 ml/min using a syringe pump (Sage Instruments, Boston, MA) over a period of 3 h. The microspheres which formed were harvested by filtration, washed with 2% polyethylene glycol 400 solution to reduce aggregation and remove drug particles on the surfaces, dried over calcium chloride under vacuum in a desiccator for at least 24 h, then sieve-sized into various fractions.

2.2. Determination of percent drug microencapsulated and drug loading

Microspheres (10 mg) were added to 5 ml of methylene chloride, a solvent for the polymer but

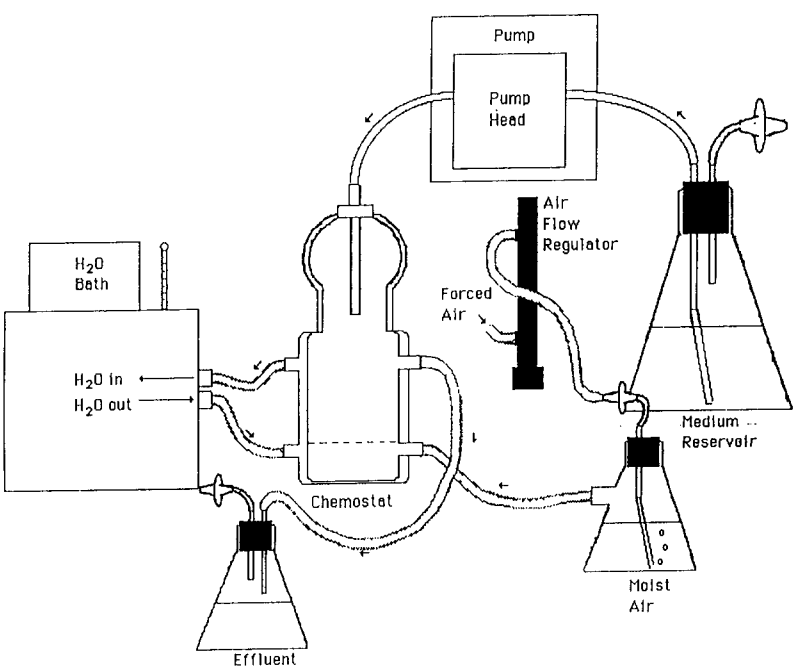

Fig. 1. Diagram of the chemostat system showing the configuration and structure of the components.

not the drug. Phosphate buffer solution (PBS) (30 ml) was then mixed with the organic solution causing the CFX or CPX particles to dissolve in the aqueous phase. The aqueous phase was separated and the extraction process repeated twice. Drug concentrations were determined spectrophotometrically at either 328 nm (CFX) or 260 nm (CPX) (Beckman, Model 25 spectrophotometer). Experiments were run in triplicate and the results averaged. The encapsulation efficiency (fraction of initial amount of drug microencapsulated) and drug loading (fraction of microspheres consisting of drug, % w/w) were determined.

2.3. The chemostat system

A diagram of the chemostat system is shown in Fig. 1. The chemostat was filled with pH 7.4 PBS as the release medium, then additional medium was pumped at a fixed rate (input) into the vessel (peristaltic pump, Watson Marlow). Simultaneously, medium flowed out of the chemostat at the same constant rate (output) to the waste tank maintaining a constant volume of 120 ml in the chemostat. Water circulation through the walls of the chemostat provided a constant temperature of 37° C. The contents of the chemostat were aerated at a constant air-flow rate of 1 l/min, which provided continuous mixing and ensured homogeneity of the contents.

2.4. Determination of drug release in the chemostat

Normally, equivalent weights of either drug powder or drug in microspheres (30 mg of CPX, 7 mg of CFX) were tested. The microspheres were sealed in a polyethylene screen (Spectra/Mesh, 110 μm mesh size, Fisher Scientific) to retain them in the chemostat. Medium was pumped into the chemostat at a constant rate of 0.46 ml/min unless otherwise stated. Samples of effluent were withdrawn at specified time intervals and analyzed spectrophotometrically, obtaining drug concentrations from a calibration curve. The effects of drug loading, particle size, dose, and PBS delivery rate on the release profiles were examined.

3. Results

The microspheres were approximately spherical under the optical microscope and were free-flowing. Separation of a batch of microspheres into various sieve-sized fractions was easily performed. Typically, under the stated conditions, 42.1% (0.6) (SD in parentheses), 33.4% (0.8), 24.6% (1.0) of a batch of CPX microspheres, and 37.1% (1.2), 24.0% (1.4), 39.0% (1.2) of a batch of CFX microspheres (by weight) were separated into 250–425, 125–250, and < 125 μm sizes, respectively. The percent drug encapsulated for each loading was consistently > 90%.

Fig. 2 and 3 illustrate the release profiles and dissolution rate of CPX and CFX, respectively. The solubility of CPX in pH 7.4 PBS was determined to be 40 mg/ml at 37° C (cf. 40 mg/ml, Griffith and Black, 1970). The peak concentration of CPX from powder was 225 μg/ml, obtained (from 30 mg) after about 5 min. Release of CPX from 33 and 43% w/w PLA-loaded microspheres in the open chemostat system yielded peak drug concentrations of about 150 and 175 μg/ml which were sustained for about 90 and 30 min, respectively. Consequently, concentrations from microspheres after 6 h were twice those produced from dissolution of the powder.

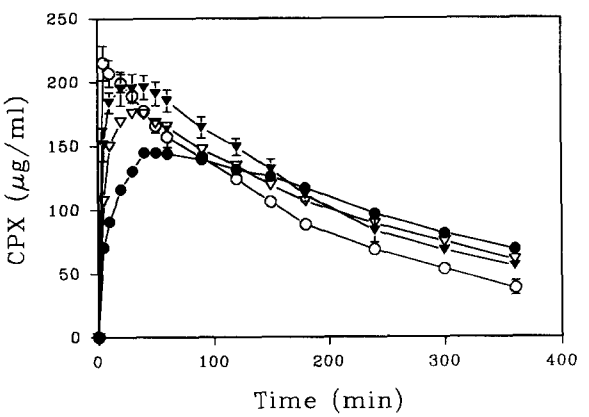

Fig. 2. Release of 30 mg equivalent CPX from PLA (250–425 μm) microspheres in the chemostat at 37° C, pH 7.4 PBS at a dilution rate of 0.46 ml/min as a function of drug loading. (●) 33% w/w; (▽) 43% w/w; (▼) 33% w/w, 125–250 μm microspheres; (○) dissolution of CPX powder.

In contrast, CFX has a solubility of 5 mg/ml at pH 7.4 and its dissolution profile (from 7 mg) indicated a slower rate of dissolution than CPX (Fig. 3). The peak concentration of CFX was 30 μg/ml, reached after about 40 min, followed by a gradual decrease in the chemostat concentration reaching 10 μg/ml after 6 h, a 67% drop from the peak concentration (cf. 77% drop for CPX).

The concentration of CFX obtained from PLA microspheres at a 0.46 ml/min flow rate increased then plateaued. The higher the drug loading, the higher was the plateau level of CFX. Thus, concentrations of 14, 18, and 22 μg/ml of CFX were maintained by microspheres of 33, 50, and 71% w/w drug loading, respectively, in the open chemostat system for at least 6 h.

The effects of varying the microsphere mean size on the release of CPX and CFX from PLA microspheres are also shown in Fig. 2 and 3, respectively. Microspheres of CPX in the 125–250 μm size range produced a peak CPX concentration very similar to that obtained from the dissolution of CPX powder (about 200 μg/ml) but only after about 30 min (cf. 5 min for the powder). Also, the peak concentration from the microspheres was sustained for about 45 min (the peak concentration of CPX powder was not sustained). In comparison, using 250–425 μm sized PLA microspheres the peak concentration of CPX was

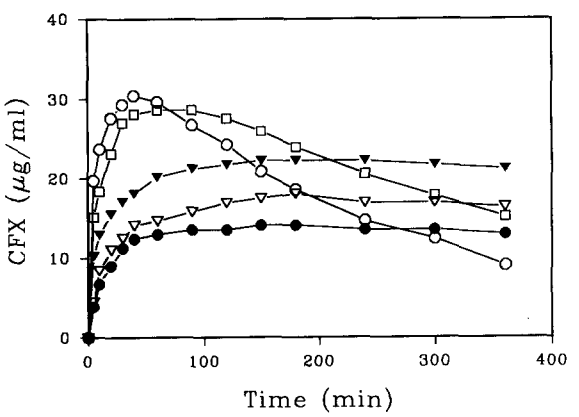

Fig. 3. Release of 7 mg equivalent CFX from PLA microspheres in the chemostat at 37° C, pH 7.4 PBS at a dilution rate of 0.46 ml/min as a function of drug loading. (●) 33% w/w; (▽) 50% w/w; (▼) 71% w/w; (□) 71% w/w, 125–250 μm microspheres; (○) dissolution of CFX powder.

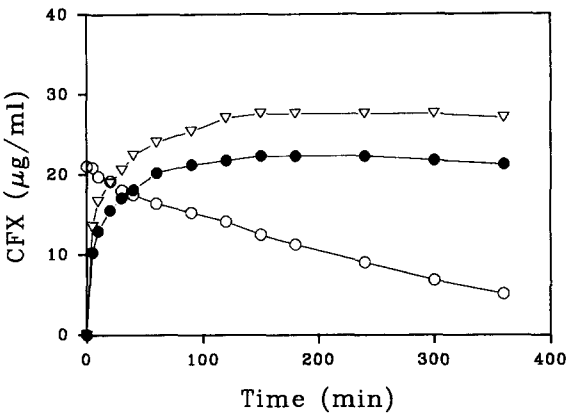

Fig. 4. CFX release from 71% w/w PLA microspheres in the chemostat at 37° C, pH 7.4 PBS at a dilution rate of 0.46 ml/min as a function of the dose administered. (●) 7 mg; (▽) 10 mg; (○) clearance rate of CFX from a 22 μg/ml solution.

150 μg/ml, reached after about 45 min, which was sustained for approx. 1 h.

CFX powder and microspheres in the 125–250 μm size range also yielded similar CFX peak concentrations at 30 μg/ml (Fig. 3), but concentration levels were sustained from the microsphere formulation for approx. 1.5 h compared to only 30 min from the powder. This compares with concentrations of CFX from microspheres in the 250–425 μm size range which increased gradually and plateaued at 22 μg/ml after about 2 h, and which were maintained in the chemostat for at least 6 h under the flow-through conditions.

Fig. 4 represents the effect of dose on the concentration-time profiles in the open chemostat system. A 7 mg dose or a 10 mg dose of CFX in microspheres resulted in similar profiles, except the steady-state concentration derived from the 10 mg dose was 27% higher (28 vs 22 μg/ml). In contrast, concentration levels diminished with time to about 5 μg/ml after 6 h for an initial 22 μg/ml solution of CFX in the chemostat.

By adjusting the pump speed, the input/output rate of medium in the chemostat could be varied. It can be seen in Fig. 5 and Table 1 that decreasing the rate from 0.46 to 0.23 ml/min yielded a higher plateau concentration of CFX of 25 μg/ml but at a later time (2.5 vs 2 h), whereas increasing the rate to 0.75 ml/min produced a

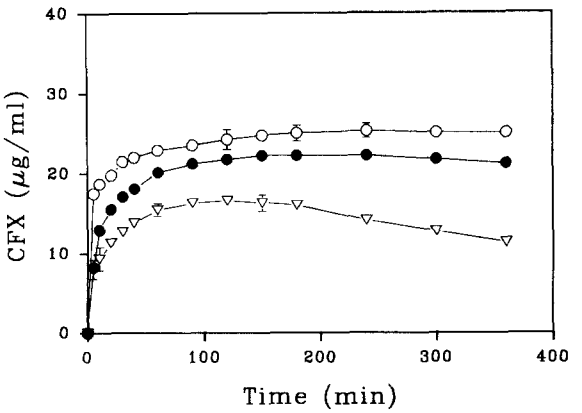

Fig. 5. CFX release from 71% w/w PLA microspheres in the chemostat at 37° C, pH 7.4 PBS as a function of the dilution rate. (○) 0.23 ml/min; (●) 0.46 ml/min; (▽) 0.75 ml/min.

lower plateau concentration of 16 μg/ml, reached after 1.5 h which was maintained for 2 h, after which the concentration gradually decreased to 11 μg/ml at 6 h.

4. Discussion

The open chemostat represents a standard first-order kinetic model for the elimination of an initial concentration of drug in solution. However, when a dosage form is included a new input parameter is introduced due to either a dissolution step or a release phase, depending on the dosage form. In the case of fast dissolution, a peak drug concentration occurs after a short time then drug concentration decreases according to the kinetics established in the model. Such is the case with CPX because of its relatively high aqueous solubility (Fig. 2). In the case of CFX, which had a slower dissolution rate (Fig. 3), the peak occurred at a lower concentration and concentrations fell more gradually thereafter. When a microsphere formulation was used, the rate of drug input was less but concentrations were sustained more for CFX than CPX, correlating with their solubilities. Increasing the drug loading or decreasing the mean microsphere size had the effect of sustaining drug concentrations at higher levels, because of the corresponding higher rates of release. Since the emphasis was on describing the performance of the open chemostat no attempt was made to improve the yield of any particular sieve-sized fraction of drug.

The dilution rate of 0.46 ml/min in the chemostat was selected because the $t_{1/2}$ of drug elimination closely approximated the reported biological $t_{1/2}$ for CPX (Nightingale et al., 1975) and CFX (Lebel, 1988), and is a much slower rate than normally employed in flow through dissolution studies (Nicklasson et al., 1991). However, other dilution rates may be appropriate to correspond to different sites of administration. Hence, a comparison of the effect of different dilution rates in the chemostat on the kinetic profiles could aid in selecting the formulation having the most favorable release kinetics. For example, 71% w/w microspheres could sustain CFX concentration levels for several hours depending on the dilution rate.

The release profile of CFX from 71% w/w microspheres studied for 24 h in the open chemostat is compared in Fig. 6 to that obtained in a spin-filter dissolution test apparatus (a closed system) containing 500 ml dissolution medium. It is apparent that although the initial rates of release are different in the two systems because of the different rates of mixing of the microspheres, the open chemostat gives more appropriate informa-

Table 1
Kinetic parameters of CFX release from 71% w/w PLA microspheres in the open chemostat as a function of the flow rate

Flow rate (ml/min)	C_{max} (μg/ml) [a]	T_{max} (h) [b]	Duration of C_{max} (h)
0.23	25.0	2.5	4.0
0.46	22.0	2.0	3.0
0.75	16.0	1.5	2.0

[a] Maximum CFX concentration in the chemostat after a 7 mg dose of CFX.
[b] Time to reach C_{max}.

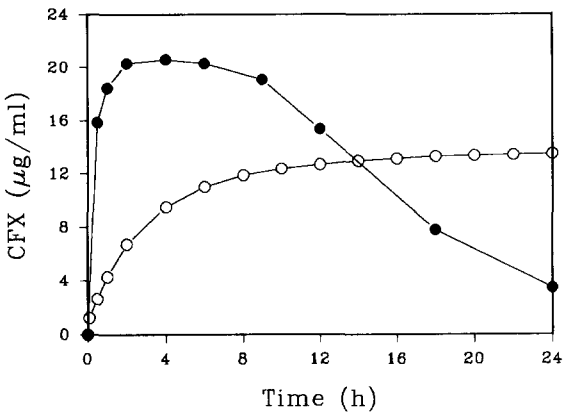

Fig. 6. Comparison of the 24 h release profiles of 7 mg equivalent CFX in 71% w/w PLA microspheres in the: (●) open chemostat (120 ml constant volume and 0.46 ml/min flow rate); (○) spin-filter dissolution test apparatus (100 rpm, 500 ml dissolution medium).

tion with respect to the in vivo situation. Concentrations in the dissolution medium of the spin-filter apparatus approached the theoretical concentration (14 μg/ml) after approx. 90% of CFX had been released from the microspheres. On the other hand, the peak concentration in the open chemostat reached 20 μg/ml, which was only about 35% of the theoretical concentration (58 μg/ml) if the medium in the chemostat was not being diluted as a function of time. Recognizing, for example, that it is important to be able to predict which formulation of CFX wll render a sustained concentration above the MIC (or BEC) of an antibiotic against a bacterium growing on a peritoneal implanted catheter, the chemostat is more appropriately designed to allow selection of the best formulation and the frequency of dosing for optimum performance.

When a sustained release formulation of a drug is injected, for example, in the peritoneum, the rate of drug clearance from the peritoneum will be governed by the rate of release from the formulation and the rate of absorption (and metabolism). Sustained fluid levels of drug are achieved when the formulation has been designed to release drug (input) at a rate that counterbalances the clearance (output) rate. Drug solubility and particle size are important factors in the dissolution process while drug solubility, polymer composition, polymer molecular weight, microparticle size and drug loading are important factors in sustained release. In developing a strategy to determine the optimum dose in vivo, an in vitro test method which can reveal this for any given formulation would be advantageous. The flow-through behavior of the open chemostat appears to possess this capability as demonstrated with antibiotic microspheres while being versatile for potential investigation of other formulations. The open chemostat does not have problems of clogging, pressure build-up, or inadequate flow rates associated with some flow through systems (Abdou, 1990; Washington and Koosha, 1990). Advantages of the flow-through cell method over the USP Paddle or Basket methods for the dissolution of microparticulate systems have been shown (Nicklasson et al., 1991). Chemostats can be connected in series to facilitate the release studies and the release medium leaving the effluent port of the chemostat can be routed through a spectrophotometer for automatic recording of absorbances. A pH gradient can also be set up to study drug release under changing pH conditions.

The dilution rate of the drug in the chemostat can be adjusted to simulate the clearance of drug in vivo which forms a basis for comparison of formulations. The intraperitoneal route of administration is particularly appropriate for formulations characterized by open chemostat studies because local concentrations of antibiotic maintained above the MIC for a sufficiently long period of time are required to treat infections in the peritoneum. In this case, selection of the microsphere size and loading that provides sustained concentrations of antibiotic can be made from chemostat studies once the pharmacokinetics of the drug in the peritoneum are known. It is then a simple matter of adjusting the flow rate in the chemostat and developing formulations which produce sustained concentrations of drug at a level appropriate for effective therapeutic treatment. It is suggested that the use of the chemostat in developing new sustained release formulations could also reduce the requirement of animals to define the relevant parameters.

Acknowledgements

The authors are grateful to the Medical Research Council of Canada and the Canadian Commonwealth Scholarship Fund for financial assistance.

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