Development of an in Vitro Dissolution Method Using Microdialysis Sampling Technique for Implantable Drug Delivery Systems

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Abstract □ The major challenge faced during the development of implantable dosage forms for site-specific delivery is monitoring the local concentration of the drug at or around the site of action. The tissue concentration at the site is generally measured by either sacrificing the animal at different points in time or by determining the amount of drug left in the implants at various time intervals. Unfortunately, there are no official in vitro dissolution methods available to study the release characteristics of drugs from this drug delivery system. The objective of this investigation was to develop a simple method using microdialysis sampling technique to serve as an in vitro dissolution method for implantable drug delivery systems. Ciprofloxacin implants were prepared by compressing ciprofloxacin microcapsules in poly(lactic acid) (PLA) and poly(lactic-glycolic acid) (PLGA). A sensitive HPLC method was developed and validated for the assay of Ciprofloxacin. An in vitro dissolution method was developed to study the release characteristics of drug from these implants. The method used a microdialysis sampling technique and a small sample volume of release medium. The various advantages and disadvantages of this method over other USP methods are discussed.

Introduction

In vitro dissolution testing of pharmaceuticals is not a guarantee of therapeutic efficacy, but it is the best available in vitro method that can reveal qualitatively the physiological availability of a drug. Furthermore, the FDA Generic Drugs Advisory Committee has recently recommended the use of dissolution testing as an in vitro surrogate marker for bioavailability and bioequivalence.[1] Unfortunately, no such methods are officially available for implantable dosage forms. An in vitro method which may correlate to the local concentration of drug near the target site(s) will be invaluable for the design and evaluation of implantable dosage forms. Implantable dosage forms are gaining tremendous interest for the treatment of bone cancer, osteomyelitis, and for the delivery of short half-life polypeptides.[2−4] Therefore, a need to develop an in vitro dissolution method for these dosage forms will be an important contribution to the area of pharmaceutical dosage form design and analysis.

Microdialysis is a very useful technique because it permits continuous monitoring of drug concentration in extracellular spaces and has been used extensively in pharmacokinetics and pharmacodynamics studies.[5−7] In vitro microdialysis sampling technique has also been used to determine the protein binding of a drug,[8,9] partition coefficient,[10] and dissolution testing of pharmaceutical formulations.[11,12] The overall goal of this study is to develop an in vitro dissolution method using a microdialysis sampling technique to study the release characteristics of drugs from implantable dosage forms and compare the in vitro release profiles obtained from an established USP dissolution method. The idea of microdialysis is to mimic the passive function of a capillary blood vessel by perfusing a perfusate solution through a thin dialysis tube implanted in the tissue near the site of the implant. Therefore, the objectives of this investigation are to (i) develop and validate a sensitive HPLC method for the assay of ciprofloxacin, (ii) design a biodegradable implant for ciprofloxacin, (iii) design an in vitro dissolution apparatus using microdialysis as the sampling technique to study the release characteristics of ciprofloxacin from the implants, and (iv) compare the in vitro release profiles of drugs obtained from this method with an established USP dissolution method.

Experimental Section

Materials—Ciprofloxacin (Miles Pharmaceutical, West Haven, CT), a broad-spectrum antibiotic of the fluoroquinolone group, was used as a model compound. Poly DL-lactide-*co*-glycolide (PLGA) (50:50) and poly-lactic acid (PLA) (Birmingham Polymer, Birmingham, AL) were used as the biodegradable polymeric matrix materials. Citric acid, sodium phosphate (dibasic), acetonitrile, methanol, perchloric acid, and water (HPLC grade) were used as received (Fisher Scientific, Fair Lawn, NJ).

Preparation of Microcapsules and the Implants—Coacervation-phase separation method was used for the preparation of the microcapsules. The PLGA/PLA polymer (1 g) was dissolved in 30 mL of methylene chloride. Ciprofloxacin hydrochloride powder was dispersed in 600 mL of cyclohexanes. The polymer solution was then added, dropwise, over a period of 5 min to the cyclohexanes containing the dispersion of the drug. Methylene chloride was then evaporated off over a period of 2.5 h. After the microcapsules were formed, the solution was vacuum-filtered and dried overnight. Implants were made by compressing ciprofloxacin microcapsules in a Carver press using stainless steel dies and punches. The compression force used was 1000 psi for 5 s.

Scanning Electron Microscopy (SEM)—Samples were mounted onto the SEM specimen stub using transparent adhesive tabs. They were coated with gold and palladium, for 3 min, using a Polaron (model-E511) sputter coater and examined under a JEOL-JSM840 SEM (JEOL, Tokyo, Japan) operated at 10 kV.

Assay of Ciprofloxacin—The HPLC method developed by Bauer et al. was extensively modified and used for the analysis of the drug.[13] The mobile phase consisted of a citrate buffer: acetonitrile:methanol mixture (85:10:5 v/v/v). The apparent pH of the mobile phase was adjusted to 2.4 with perchloric acid. The citrate buffer (pH 3.8) was prepared by mixing 64.6% (v/v) of 0.1 M citric acid to 35.4% (v/v) 0.2 M Na_2HPO_4 solution. The flow rate was maintained at 1.5 mL/min (Shimadzu LC-6A pump). A Spherisorb C18 pH stable column (Phase Separations, Norwalk, CT), 15 cm in length, was used, and the column effluents were monitored at 280 nm (Shimadzu SPD-6A UV detector, Shimadzu. Koyoto, Japan).

Microdialysis System—This system consisted of a Harvard-22 syringe pump, Hamilton gastight (3.26 mm diameter) 500 μL

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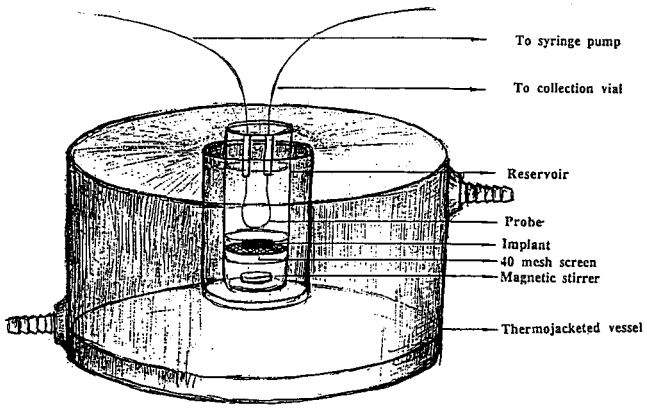

Figure 1—In vitro dissolution apparatus with microdialysis sampling technique designed in our laboratory for implantable drug delivery system.

syringes, and flexible microdialysis probes (made in house).[8] Flow-through loop-type probes were constructed using fused-silica tubing (Polymicro Technologies, Phoenix, AZ) with 75 μm i.d. and 150 μm o.d. Regenerated cellulose Spectro/Pro microdialysis hollow fibers (Spectrum Medical Industries, Los Angeles, CA) with a molecular weight cut-off of 1300 Da and 28−36 mm long were used as the dialysis membrane. The inner diameter of the dialysis fiber was 150 μm with a wall thickness of 9 μm. Probes were connected to the perfusion syringe with fused silica tubing. Initially, the probes were soaked and perfused with 80% (v/v) ethanol in water. Prior to experimentation, all of the probes were soaked and perfused with Sorensen's phosphate buffer (pH 7.4). These experiments were carried out at 37 °C with constant stirring (50 rpm) of the dissolution medium.

In Vitro Dissolution Apparatus with Microdialysis Sampling Technique—An in vitro dissolution apparatus which utilizes a microdialysis sample collection technique was developed in our laboratory and a schematic representation of the apparatus is depicted in Figure 1. The diameter of the reservoir was 15 mm, and its height was 45 mm. A 40 mesh screen was kept at a height of 5 mm from the bottom of the reservoir. The implantable delivery system was placed on the screen, and the microdialysis probe was placed at a fixed distance (10 mm) from the implant. Sorensen's phosphate buffer (pH 7.4) was used as the perfusate and the dissolution medium. The release medium was constantly stirred with the aid of a magnetic stirrer (50 rpm) placed at the bottom of the release medium as shown in Figure 1. The flow rate of the perfusate varied from 0.5 to 1 μL/min. At 25−40 min time intervals, the perfusate samples were collected directly into the HPLC autosampler injection vials with 200 μL inserts. To avoid evaporation, the vials were rapped with Parafilm. After the end of sample collection the Parafilm was immediately replaced with the cap containing the rubber septum. The concentration of the drug in the perfusate was determined using the HPLC method.

Microdialysis Probe Calibration—The in vitro calibration was performed at 37 °C in a Sorensen's phosphate buffer in a special thermojacketed Plexiglass microdialysis chamber containing 8.0 mg/L of ciprofloxacin. Probe recovery was measured before each experiment.

Factors Affecting the in Vitro Microdialysis Recovery—Probe flow rate was validated gravimetrically using preweighed collection vials. The effect of the flow rate and probe length on recovery of the drug during the microdialysis studies were evaluated.

Data Analysis—The in vitro recovery of the probes was calculated as the ratio of the ciprofloxacin concentration in the dialysate to that in the Sorensen's phosphate buffer in the reservoir vial, expressed as the peak height ratio (PHR) of drug to the internal standard (IS), represented by eq 1:

$$\text{recovery}_{\text{in vitro}} = \frac{C_{\text{dial}}}{C_{\text{res}}} = \frac{\text{PHR}_{\text{(drug/IS) dialysate}}}{\text{PHR}_{\text{(drug/IS) reservoir}}} \qquad \text{(eq 1)}$$

USP Dissolution Apparatus 3[14]—Sorensen's phosphate buffer pH 7.4 (150 mL) was used as the dissolution medium and equilibrated to 37 °C. The ciprofloxacin implant was then placed on the plastic wire mesh of the reciprocating tube and dipped at a rate of 20 dips per minute into the dissolution medium. At predetermined time intervals, 1 mL of the sample was collected and an equal volume of fresh phosphate buffer was replaced after each sample collection. The drug content in the release medium was determined by the HPLC method.

Results and Discussion

Formulation of the PLGA Implants Containing Ciprofloxacin—The drug is highly water soluble and has been reported to be stable in solution.[15] The pK_as of ciprofloxacin are 6 and 8.8 with a molecular weight of 385.8 (g/mol). These physicochemical properties indicate that the drug can easily pass through the microdialysis membranes used in this investigation and, therefore, be utilized as the model drug. Initial attempts to make the PLGA/PLA implants by direct compression of the physical mixture of the drug and polymer in a Carver press was unsuccessful. Therefore, the drug was first microencapsulated in the polymer and then compressed into cylindrical implants. The microstructure of the drug both before and after microencapsulation is shown in Figure 2. The scanning electron micrographs indicated that the free drug (Figure 2a) is crystalline and rod-shaped in nature, whereas the microcapsules (Figure 2b) are agglomerates with a different microstructure. Such a change in microstructure was essential for the direct compression of these polymers (PLA and PLGA) and the drug (ciprofloxacin) into implant. The detailed dimensions of the implants made and their composition are provided in Table 1.

HPLC Method Validation—Representative chromatograms of the internal standard (phenacetin), ciprofloxacin, and both the drug and internal standard are shown in Figure 3. No interfering peaks were observed in the chromatograms. The linearity, precision, accuracy, and sensitivity of the assay were determined as indicated below.

Linearity—The standard curves were linear over the concentration range of 0.0−9.5 mg L^{-1}. The equation of the standard curve relating the peak height ratio (P) to the ciprofloxacin hydrochloride concentration (C in mg l^{-1}) in this range was:

$$P = 0.27578C + 0.00147, \qquad r^2 > 0.999$$

Precision—Within-day precision was determined by analysis of four different standard curves on the same day. Day-to-day precision was determined from the standard curves prepared on each seven different days during 60 days. The precision of the assay was determined from the variability in the peak height ratio at each concentration. The RSD for the within-day and day-to-day precision were 0.6−2.6% and 1.9−4.3%, respectively, and are depicted in Table 2.

Accuracy—Known amounts of ciprofloxacin were added to the mobile phase to make the quality control samples. Three quality control samples and the standard solutions were kept at 4 °C for 60 days. These solutions were analyzed seven times during this period, and the accuracy of the assay was determined by comparing the measured concentration to its true value of the drug. The RSD for the accuracy measurement for this method was between 3.8 and 4.7% as shown in Table 3.

Sensitivity—The sensitivity criteria was determined from seven different standard curves using the lowest limit of reliable assay measurement guidelines described by Oppenheimer et al.[16] In this study, the critical level is defined as the assay response above which an observed response is reliably recognized as detectable. The critical level is also considered a threshold value, thus, defining

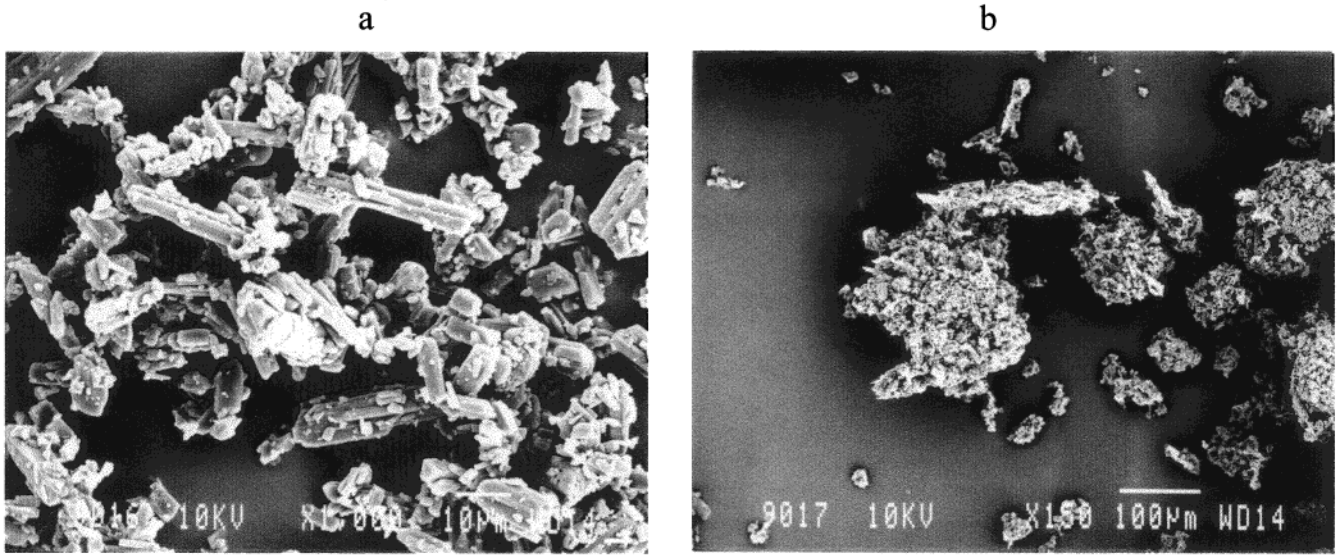

Figure 2—Scanning electron micrographs of (a) Ciprofloxacin powder, and (b) microencapsulated Ciprofloxacin in PLGA.

Table 1—Physical Dimensions and Drug Load of the Implants

implant ID	polymer used	weight of implant (mg)	height of the implant (mm)	diameter (mm)	actual[a] drug load, % (w/w)
A	PLGA	220.0	7.0	6.0	9.4
B		216.4	6.5	6.0	
C		216.7	6.6	6.0	
A	PLA	135.3	3.5	6.0	9.6
B		144.5	4.0	6.0	
C		144.3	4.0	6.0	
A	PLA	152.7	4.0	6.0	16.6
B		153.8	4.0	6.0	
C		154.1	4.0	6.0	

[a] Actual drug load represents the drug load determined by HPLC analysis.

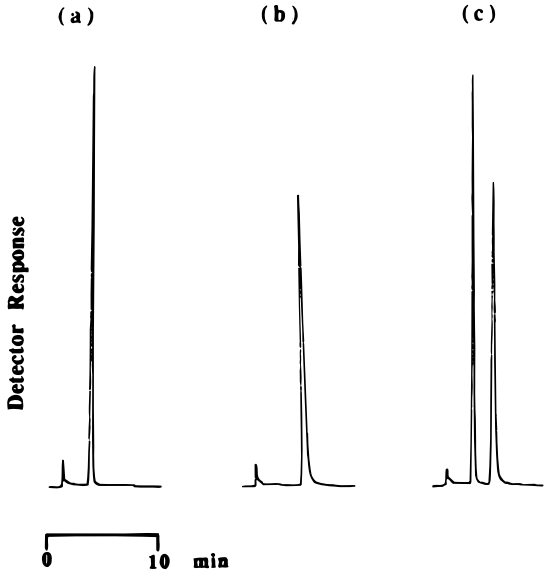

Figure 3—Representative chromatograms obtained following injection of (a) Phenacetin (21.3 mg L^{-1}), (b) Ciprofloxacin (3.9 mg L^{-1}), and (c) Phenacetin (21.3 mg L^{-1}), and Ciprofloxacin (3.9 mg L^{-1}).

detection. Therefore, if the measured response exceeds this value, the presence of an analyte is detected, otherwise, it is not reliably recognized as detectable. In this investigation, the critical value was determined as 0.034 ± 0.007 μg mL^{-1} (mean ± SD).

Table 2—Within-Day and Day-to-Day Analytical Precision of Ciprofloxacin Assay

	within-day[a]		day-to-day[b]	
concn (mg L^{-1})	peak height ratio[c]	RSD (%)	peak height ratio[d]	RSD (%)
0.00	0.000	–	0.000	–
0.19	0.046 ± 0.001	2.6	0.050 ± 0.002	4.3
1.90	0.545 ± 0.008	1.3	0.520 ± 0.010	1.9
2.85	0.734 ± 0.004	0.6	0.780 ± 0.030	3.8
5.7	1.511 ± 0.041	2.7	1.590 ± 0.030	1.9
7.6	1.987 ± 0.048	2.4	2.09 ± 0.070	3.3
9.5	2.579 ± 0.053	2.1	2.620 ± 0.080	3.1
slope	0.267 ± 0.0022	0.81	0.276 ± 0.0072	2.6

[a] Analyzed on the same day. [b] Analyzed on seven different days within a period of 60 days. [c] Mean ± SD; $n = 4$. [d] Mean ± SD; $n = 7$.

Table 3—Accuracy in the Analysis of Ciprofloxacin in Quality Control Samples

actual concentration (mg L^{-1})	accuracy[b]	RSD (%)
0.95	99.82 ± 4.7[a]	4.68
3.80	99.59 ± 3.8	3.78
6.65	100.75 ± 4.5	4.45

[a] Mean ± SD; $n = 7$. [b] Accuracy = (measured concentration/actual concentration) × 100.

In addition to the critical value, another important parameter is the detection level defined as the actual net response, which may, a priori, be expected to lead to detection. This response is defined as the smallest value of the true concentration that is "nearly sure" to produce a measurement value that results in detection.[17] The detection level in this analysis was found to be 0.069 ± 0.013 μg mL^{-1} (mean ± SD).

The determination level is defined as the concentration at which the measurement precision will be satisfactory for quantitative analysis. This level was determined as 0.191 ± 0.035 μg mL^{-1} (mean ± SD) to obtain a 10% RSD level of precision.

Microdialysis Probe Calibration—The recovery of the probes was determined prior to each in vitro release study. The effects of probe length and perfusion flow rate on the in vitro recovery of ciprofloxacin were then determined and shown in Table 4. As expected, decreasing the length of the probe decreased the in vitro recovery of the drug.

Table 4—Effect of Probe Length and Flow Rate on the in Vitro Recovery of Ciprofloxacin

probe length (mm)	flow rate (μL/min)	mean recovery ± SD[a] (%)	RSD (%)
36	0.75	83.47 ± 2.5	1.19
31	0.75	80.64 ± 1.6	1.98
29	0.75	77.42 ± 0.33	0.43
28	1.0	56.52 ± 2.23	3.95
28	0.75	73.64 ± 3.96	5.38
28	0.5	79.98 ± 4.90	6.12

[a] Mean ± SD; $n = 3$.

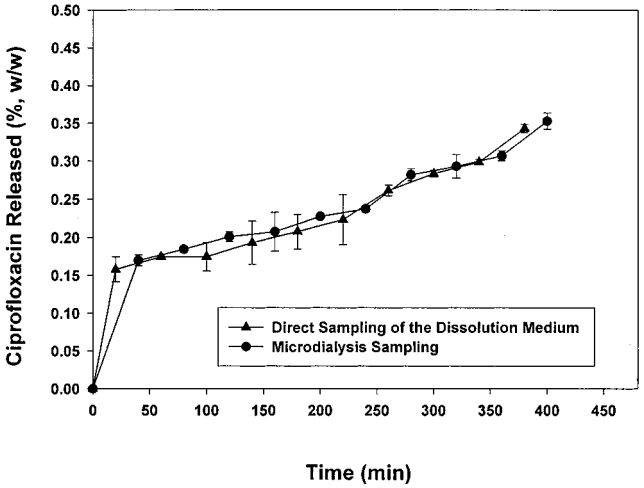

Figure 4—In vitro release profiles of Ciprofloxacin from PLGA implants (9.4% w/w drug load) determined by direct sampling of the dissolution medium and sampling by microdialysis.

However, the perfusate flow rate had an inverse relationship with the in vitro recovery of the drug. Through the analysis of the results of these studies, the optimal perfusate flow rate was determined to be 0.75 μL/min and the length of the microdialysis probe was kept within the range of 30−32 mm for the entire study. Probe flow rate was validated gravimetrically using preweighed collection vials. Within sample precision of the microdialysis sampling was determined in seven microdialysate samples collected using the same probe and same flow rate. The RSD for this precision determination was within 5%.

In Vitro Release of Ciprofloxacin from the Implants—The in vitro release of ciprofloxacin from these implants was then determined by two separate and independent methods. The drug was first analyzed using the method developed in our lab with microdialysis sampling technique and second by utilizing USP dissolution method 3.

Using the dissolution method developed in our lab, the ciprofloxacin concentration in the release medium was determined by two different sampling methods. At appropriate time intervals, 30 μL of the sample was collected directly from the dissolution medium and injected into the HPLC for assay of ciprofloxacin. Simultaneously, 30 μL of fresh phosphate buffer was replaced in the chamber. In the second sampling method, the microdialysis probes were used to monitor the concentration of the drug in the dialysis chamber. Before using the probes for monitoring the in vitro drug concentration, they were calibrated to determine their in vitro recovery. The perfusate (30 μL) was collected every 40 min at a flow rate of 0.75 μL/min, and the concentration of the drug in the microdialysate was determined by HPLC. The drug concentration in the dissolution medium was then calculated using the in vitro recovery of the probe. Figure 4 depicts the in vitro release profiles of

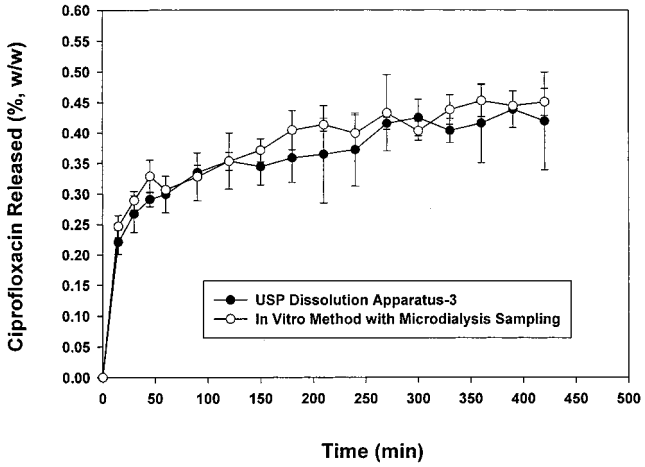

Figure 5—Comparison of the in vitro release profiles of Ciprofloxacin from PLGA implants (9.4% w/w drug load) determined by the use of microdialysis sampling method and the USP dissolution method 3.

Table 5—Comparison of the Extent of Ciprofloxacin Released in 6 h from Various Implants with Similar Drug Load. The in Vitro Release Profiles of PLGA Implants Are Only Shown in Figure 4 and Figure 5

implant type	microdialysis using direct sampling	microdialysis using microdialysis probe	USP apparatus 3
PLA	0.15 ± 0.003[a]	0.15 ± 0.004	0.17 ± 0.02
PLGA	0.40 ± 0.01	0.44 ± 0.05	0.41 ± 0.02

[a] Mean ± SD; $n = 3$.

the drug from a PLGA implant determined by the two sampling techniques. The method by which the samples were collected had no effect on the release profiles of the drug. Therefore, this study clearly indicated that this microdialysis sampling technique could be used to monitor the concentration of various drugs in the release medium if the determination of the in vitro recovery of the probe is correct.

The in vitro release characteristics of ciprofloxacin from the implants were then studied using the two independent methods. We selected the USP dissolution method 3 for the following reasons: (i) a low volume (150 mL) of release medium is required as compared to the 900 mL in the case of USP methods 1 and 2, (ii) low rate of evaporation of the release medium during dissolution studies over a prolonged period of time as compared to the USP methods 1 and 2, and (iii) in our laboratory, we have already compared and reported the in vitro release characteristics of other dosage forms using both USP apparatus 2 and 3 without any significant differences in their release profiles.[18] The in vitro release profiles of the drug from various implants containing similar drug loads were then obtained by both methods and are shown in Figure 5. The rate and extent of drug release determined by both of these methods were in close agreement. The burst effect shown during the in vitro release studies could arise due to the presence of free drug on the surface of the implants. This free drug might have originated during compaction of the microcapsules or due to the presence of uncoated drug in the formulation. Table 4 summarizes and compares the extent of ciprofloxacin released from various implants with similar drug loads in 6 h. This study indicated that the release of ciprofloxacin from the PLGA implants was higher than the PLA implants (release profiles not shown here). Moreover, the preparation of PLGA microcapsules was easier than the PLA microcapsules. The former microcapsules were compact, whereas the latter were fluffy and difficult to handle during compression. Comparison of the in vitro

release profiles of drug from the implants within a batch was found to be in close agreement and independent of the sampling (Figure 4) and dissolution methods (Figure 5) used. However, a slight difference in the release profiles was noticed between batches (Figure 4 and Figure 5) even though the drug load was kept constant in both the batches. This difference in the release profiles could possibly be attributed to batch-to-batch variations.

Conclusions

(1) A sensitive HPLC method was developed and validated for the analysis of ciprofloxacin.

(2) An in vitro dissolution method using a microdialysis sampling technique was developed and used to determine the release characteristics of ciprofloxacin from various implants.

(3) The proposed method has the following advantages: (i) it is simple, (ii) it requires a very small volume of dissolution medium, (iii) on-line analysis of the drug is possible, (iv) continuous monitoring of the drug over a prolonged period of time is possible, and (v) this method could be used to determine the local concentrations of the drug near the implant site.

(4) The rate and extent of ciprofloxacin release determined by both methods were in close agreement.

(5) In the future, this method could be used in evaluating the in vitro dissolution characteristics of potent drugs and dosage forms (microcapsules and microspheres of proteins and peptides) requiring a small volume dissolution medium.

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