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ScienceDirect
International Journal of Pharmaceutics 356 (2008) 206–211
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

In situ fiber optic method for long-term in vitro release testing of microspheres

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Received 4 October 2007; received in revised form 5 January 2008; accepted 8 January 2008
Available online 19 January 2008

Abstract

The objective of this study was to develop an in vitro release method for relatively unstable drugs in long-term modified release (MR) formulations, such as microspheres. Drug stability in the release medium can complicate in vitro release testing of such delivery systems. To overcome this problem, a method has been developed where the model drug, cefazolin, and its degradation products are monitored simultaneously, using UV fiber optic probes, to account for cumulative drug release from poly(lactic-*co*-glycolic) acid (PLGA) microspheres. United States Pharmacopeia (USP) Apparatus 2 and 4 were used to evaluate cefazolin release throughout the 30-day study period. Cefazolin exhibits an isosbestic point (wavelength where the drug and the degradation products have the same absorbance). Cumulative drug release was compared at the isosbestic (288 nm) point and at the UV max (270 nm). Monitoring at the isosbestic point allowed determination of total drug release with approximately 100% release by day 25. Whereas, at the UV max approximately 61% release was detected by day 25 as a result of drug degradation. Problems were encountered using USP Apparatus 2 with the in situ UV fiber optic probes as a result of microsphere accumulation at and interference with the probe detection window.

Keywords: Cefazolin; Isosbestic point; Modified release; PLGA microspheres; Fiber optic probes

1. Introduction

There has been a significant amount of research into modified release (MR) parenteral dosage forms throughout the past few decades. The advantages of MR dosage forms include lower dosing frequency and hence better patient compliance; reduced adverse reactions as a result of targeted and localized drug delivery; and improved therapeutic response with more consistent and stable blood levels. MR parenteral dosage forms include dispersed systems (such as, microspheres, liposomes, nanoparticles, emulsions, and suspensions) as well as larger implantable devices which can be injected either intravenously, subcutaneously or intramuscularly and more recently combination devices such as drug eluting stents (Baker, 1987; Siepmann and Gopferich, 2001; Sahoo and Labhasetwar, 2003; Burgess and Hickey, 2005; Torchilin, 2005, 2007). Microspheres and large implantable devices are often used to achieve long-term drug release over periods of days to months. A common complication of in vitro release testing of such systems is drug degradation in the media during testing.

Drug degradation can be accounted for by calculating the drug degradation rate and correcting for this in the amount released over time (Kim and Burgess, 2002). In some cases, the degradation rate can be minimized by careful selection and optimization of the release media and its pH and ionic strength. An alternative approach, described here, is the use of in situ UV fiber optic monitoring at the isosbestic point. The isosbestic point is the wavelength at which the drug and its associated degradation products have the same absorbance (Berlett et al., 2000).

The present research is focused on in vitro release testing of MR poly(lactic-*co*-glycolic) acid (PLGA) microspheres. Drug release from PLGA microspheres is controlled by either diffusion or erosion or a combination of both. Typically the release profiles of hydrophobic drugs consist of an initial burst phase followed by a lag phase and then a secondary burst phase (Berkland et al., 2002, 2004; Wang et al., 2002; Galeska et al., 2005; Zolnik

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doi:10.1016/j.ijpharm.2008.01.017

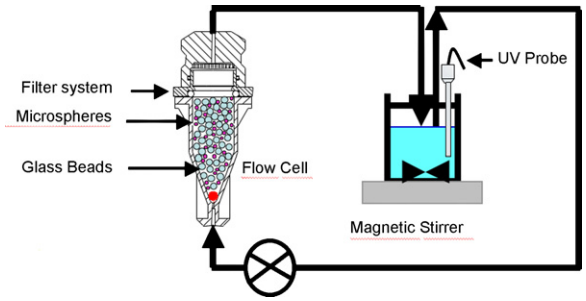

Fig. 1. Diagram of USP Apparatus 4. Reprinted with permission from Dissolution Technologies 2005.

et al., 2006). The burst phase is due to rapid release of surface associated drug, and the lag phase results from the time required for sufficient polymer degradation to facilitate drug diffusion. However, for hydrophilic drugs the burst phase is often not observed since any surface associated drug is usually removed during processing, depending on the hydrophilicity and MW of the drug.

In vitro release testing methods that have been used for MR microsphere products include USP Apparatus 1 (basket), 2 (paddle), and 4 (flow-through). USP Apparatus 1 and 2 have the complication that the media must be sampled and the microspheres separated from the media for analysis. This increases the time and manpower required for in vitro release testing, whereas in USP Apparatus 4 the microspheres are isolated within the flow-through cell (Fig. 1) and the media can be sampled from the reservoir as needed. The use of UV fiber optic probes in conjunction with USP Apparatus 1, 2, and 4 enables in situ monitoring of the release medium without the need for manual sampling. In addition, this has the advantages of frequent monitoring (as frequent as every minute); adjustable monitoring rate to allow appropriate data collection; reduced expense associated with analyst labor (Bynum et al., 2001); the entire UV spectrum can be analyzed at each time point to accrue information on degradation.

The current study utilizes in situ monitoring with fiber optic UV probes in USP Apparatus 2 and 4 to investigate cumulative drug release from PLGA microspheres. Cefazolin (Fig. 2) was chosen as a model drug since it is susceptible to degradation in aqueous media. Cefazolin is a broad spectrum antibiotic, frequently administered systemically for prophylaxis during surgical procedures to prevent infection from Gram-positive bacteria such as *Staphylococcus aureus* (Fallon et al., 1999). Cefazolin has been incorporated into polyanhydride implants

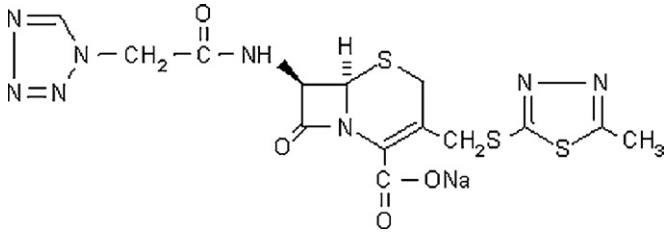

Fig. 2. The chemical structure of cefazolin sodium.

for controlled localized delivery to treat osteomyelitis (Park et al., 1998).

2. Materials and methods

2.1. Materials

Cefazolin sodium, polyvinyl alcohol (PVA), and sodium phosphate (monobasic and dibasic) were purchased from Sigma (St. Lewis, MO). PLGA resomer RG503H 50:50 (M_w: 25,000) was a gift from Boehringer-Ingelheim. Methylene chloride was purchased from Fisher Scientific (Springfield, NJ).

2.2. Methods

2.2.1. Determination of absorbance maximum and isosbestic point

Twenty milligram of cefazolin sodium was dissolved in 800 mL of phosphate buffer solution (PBS) (pH 7.4; 0.2 M). The solution was incubated at 37 °C for 30 days in a Premiere 500 Dissolution System (Distek) USP Apparatus 2 with constant stirring. Degradation of cefazolin sodium was monitored over a wavelength range of 200–400 nm, using the Type IIA UV Fiber Optic Probe dissolution system (Rainbow Dynamic Dissolution Monitor, Delphian Technology Inc, Woburn, MA). Indigo™ software package was used and data points were collected every 6 h for the first 4 days and then every 24 h.

2.2.2. Preparation of microspheres

PLGA microspheres were prepared using a w/o/w emulsion technique. Cefazolin sodium (50 mg) was dissolved in 0.625 mL of water. PLGA (1.25 g) was dissolved in 6.25 mL of methylene chloride. A w/o emulsion was prepared by mixing cefazolin solution into the organic phase (methylene chloride and PLGA) using a homogenizer PowerGen 700D Homogenizer (Fisher Scientific) at 9500 rpm for 30 s. This w/o emulsion was slowly poured into 200 mL of 0.5% (w/v) PVA solution and homogenized at 9500 rpm for 30 s. The resulting w/o/w emulsion was stirred continuously (600 rpm) at 34 °C for 5 h to allow the organic solvent to evaporate. After evaporation of methylene chloride, the PLGA microspheres were collected by filtration through 0.45 μm filter paper, washed three times with de-ionized water, and vacuum-dried overnight. Microspheres were stored at 4 °C until needed (Conti et al., 1997).

2.2.3. Preparation of standard solutions

Twenty-five milligrams of cefazolin sodium was added to 500 mL of PBS buffer (pH 7.4; 0.1 M) to prepare a stock solution of 50 μg/mL. This solution was successively diluted with PBS (pH 7.4; 0.1 M) to prepare a calibration curve (concentrations ranged from 0.66 μg/mL to 36.29 μg/mL). The Rainbow Dynamic Dissolution Monitor from Delphian Technologies, Inc. with Indigo software was utilized for analysis. The cefazolin calibration curve was calculated from the absorbances obtained at 270 nm and 288 nm, maximum absorbance and isosbestic point, respectively.

2.2.4. Microsphere characterization

2.2.4.1. Particle size measurements. Particle size distribution was analyzed using an Accusizer Model 780 Particle Sizing Systems (Santa Barbara, CA). Ten milligrams of microspheres were suspended in 2 mL of 0.1% PVA solution.

2.2.4.2. Drug loading. Cefazolin microspheres (10 mg) were dissolved in 5 mL of methylene chloride and stirred for 1 h. Ten milliliters of PBS (pH 7.4; 0.1 M) was added to the solution and stirred overnight to allow cefazolin extraction into the aqueous phase. The aqueous layer containing the drug was then analyzed using a conventional UV scan.

2.2.5. In vitro release testing with USP Apparatus 2

Premiere 500 Dissolution System USP Apparatus 2 (Distek, bathless) was used. The 800 mL vessels were filled with 600 mL of PBS buffer (pH 7.4; 0.1 M; 37 °C) and stirred at a constant rate of 100 rotations per minute. Hundred milligrams of cefazolin microspheres were added to each vessel and the vessels were covered with lids to prevent evaporation of the dissolution media. The fiber optic UV probes were placed through holes in the vessel lids and these were sealed with parafilm to prevent evaporation. All samples were scanned from 200 nm to 400 nm. Scanning intervals were set every 5 min for 1 day and then every 6 h for 4 days to monitor the initial "burst" release. The release profile was then monitored every day for the next 20 days. This monitoring schedule was selected to maximize data collection during rapid release, and minimize the file size to prevent data overload in the later stages. Prior to monitoring each time point, the probes were checked for bubbles and any bubbles present were removed. All measurements were conducted in quadruplicate and the mean values and standard deviations reported.

2.2.6. In vitro release testing with USP Apparatus 4

Sotax CE 7 smart USP Apparatus 4, consisting of a reservoir and pump for dissolution media and flow-through cells (12 mm in diameter) packed with glass beads (1 mm) (to prevent microsphere aggregation and to achieve laminar flow), was used (Zolnik et al., 2005). Whatman 0.45 glass microfiber filters were placed at the top of the flow-through cell. Fifty milligrams of cefazolin microspheres were dispersed among the glass beads in the flow-through cells. Two hundred and fifty milliliters of PBS buffer (pH 7.4; 0.1 M) was placed in the media reservoir vessels and fiber optic UV probes were placed through a three-hole rubber stopper; which prevented evaporation during monitoring. USP Apparatus 4 was set at a flow rate of 16 mL/min and the drug release media was incubated at 37 °C. All samples were scanned from 200 nm to 400 nm. The same scanning procedure and schedule as USP Apparatus 2 mentioned above was followed. All measurements were conducted in triplicate and the mean values and standard deviations reported.

3. Results

3.1. Microsphere characterization

The cefazolin microspheres had a mean particle size of 9.8 ± 1.4 μm and a drug loading of 2.39 ± 0.96% (w/w).

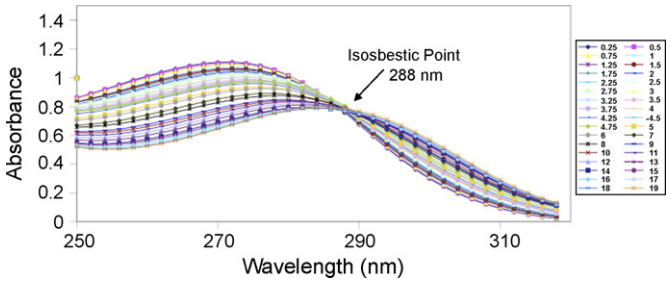

Fig. 3. The effect of time on the degradation of cefazolin sodium solution (0.2 M PBS buffer, pH 7.4, 37 °C, 30 days) monitored using fiber optic UV probes between 250 nm to 300 nm. Different time points are shown as different colors, refer to key above, times are in days. The isosbestic point of cefazolin is 288 nm.

3.2. Cefazolin UV maximum and isosbestic point

The maximum absorbance of cefazolin sodium is 270 nm (Fig. 3). However, this shifted over the 30-day study period. At day 1, the absorbance at 270 nm was 1.06 absorption units (AU) and this had reduced to 0.64 AU by day 19. The maximum absorbance peak shifted from 270 nm initially to 274 nm at day 5 and to 284 nm at day 19. These changes in the absorbance spectra were a result of cefazolin degradation. Cefazolin degradation occurs at the beta lactam moiety, where it is susceptible to hydrolytic cleavage or an intramolecular nucleophilic reaction (Yamana and Tsuji, 1976). However, at 288 nm, isosbestic point, the absorbance (0.77 AU) remained constant since at this wavelength the drug and its degradation products have the same absorbance. Standard curves were developed at both the UV max (270 nm) and the isosbestic point (288 nm) (Table 1) and both wavelengths were used for subsequent monitoring of in vitro release of cefazolin from the microspheres.

3.3. In vitro release using USP Apparatus 4

Drug release is typically measured at the UV absorbance maximum. However, for long-term releasing products such as microspheres, where drug degradation can occur in the release media it may be more appropriate to use the isosbestic point. Accordingly, drug release was evaluated at both the UV absorbance max (270 nm) and the isosbestic point (288 nm).

The cumulative release profiles at 270 nm and 288 nm started to deviate within the first day and the difference between the cumulative release at these wavelengths increased with time (Fig. 4). When monitored at 288 nm, cefazolin release from the microspheres was initially slow (first 5 days) and then followed apparent first-order kinetics with release complete around day

Table 1
Slope and intercept values from standard curves developed at both the UV maximum (270 nm) and the isosbestic point (288 nm)

	UV Max (270 nm)[a]		Isosbestic Point (288 nm)[a]	
Slope ± S.E.	0.0421	0.002	0.0296	0.001
Intercept ± S.E.	0.0056	0.021	0.0162	0.008

Solution concentrations ranged from 0.66 mcg/ml to 36.29 mcg/ml.
[a] Based on five different measurements.

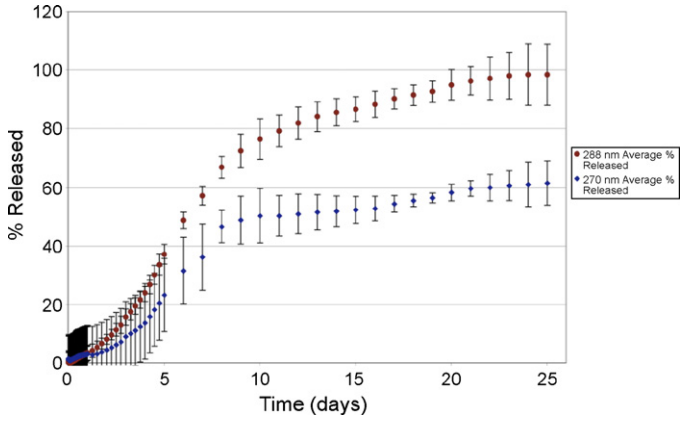

Fig. 4. Cumulative in vitro release of cefazolin sodium from PLGA microspheres using USP Apparatus 4 and measured using fiber optic UV probes at both 270 nm and 288 nm, absorbance maximum and isosbestic point, respectively. The average percent release from microspheres when monitored at the absorbance maximum was 61.4 ± 7%. The average percent release when monitored at the isosbestic point was 98.4 ± 10%.

23 (as indicated by a plateau with a total cumulative release of 98.4 ± 10%). Whereas, at 270 nm the release of cefazolin followed the same profile but appeared to be slower and the cumulative release at day 23 days was 61.4 ± 7%. The observed variation in the standard deviation may be due to the presence of bubbles on a probe that could have been missed. This problem can be solved by mechanical agitation prior to monitoring each time point.

3.4. In vitro release using USP Apparatus 2

The cumulative release of cefazolin from microspheres determined using the USP Apparatus 2 showed a similar trend to that obtained using USP Apparatus 4 (Fig. 5). The release profile at 288 nm was apparent first-order kinetics. However, the initial release rate was slower using USP Apparatus 2 and the maximum release was above 100% (109.7 ± 4%). Similarly, the release profile at 270 nm appeared to be slower than that obtained at 288 nm. In this case the cumulative release by day 25 was only 69.5 ± 5%.

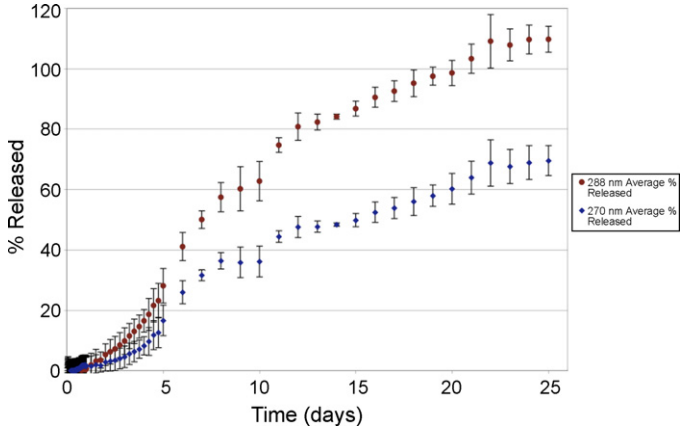

Fig. 5. Cumulative in vitro release of cefazolin sodium from PLGA microspheres using USP Apparatus 2 and measured using fiber optic UV probes at both 270 nm and 288 nm, absorbance maximum and isosbestic point, respectively. The average percent release from microspheres when monitored at the absorbance maximum was 69.5 ± 5%. The average percent release when monitored at the isosbestic point was 109.7 ± 4%.

4. Discussion

Cefazolin sodium rapidly degrades in aqueous medium and the degradation products have different UV spectra compared to the parent compound. Consequently, in vitro release monitoring at the absorbance maximum (270 nm) of cefazolin can result in erroneous data. An isosbestic point was established at 288 nm and this allows long-term UV fiber optic monitoring of in vitro release without the need for calculation of the degradation rate. Samples monitored at the isosbestic point reached 100% cumulative drug release, whereas those monitored at the UV maximum appeared to only reach approximately 60% of cumulative release. This apparent release rate is due to drug degradation. The difference between the release profiles at 270 nm and 288 nm increases with time due to the cumulative nature of drug release and the dependency of degradation on time. Therefore, drug released early in the study would be fully degraded by the end of the study. This use of UV fiber optic monitoring provides an accurate, easy and rapid method to determine in vitro drug release profiles for rapidly degrading drugs. Another method that has been used to account for drug degradation during in vitro release testing is to correct the data for drug degradation (Kim and Burgess, 2002). However, this is time consuming and complex, as degradation of released drug, unreleased drug or both may be occurring and the rates of degradation of released and unreleased drug may differ due to differences in the local environment (e.g. drug inside the PLGA microspheres can be subjected to local acidic environment). Such methods may involve determination of degradation, for example using HPLC, and it may be necessary to calculate the amount of each degradation product at each analysis time point. However, this depends on the availability of the degradation products to construct calibration curves. The degradants may be unknown and unavailable particularly for investigational drug molecules. These methods are very labor intensive and can be subjected to error, particularly for rapidly degrading drugs.

Determination of in vitro cumulative drug release has traditionally involved manual sampling and filtration of the dissolution media at predetermined time points. A major disadvantage of this method is the man-hour required for frequent samplings. To study the different phases of release throughout the entire release period, it is important to closely monitor any initial burst release phase as well as the phases that occur thereafter. Wang et al demonstrated that as the sampling interval increased from 20 min to 4 h, it becomes difficult to distinguish between the separate phases resulting in difficulties in assessing the correct release kinetics (Wang et al., 2002). In situ fiber optic UV probes enables the programming of variable sampling intervals from seconds to days to accurately monitor different release profiles from such MR microsphere formulations as illustrated in Figs. 4 and 5.

The differences observed in the cefazolin release profiles obtained using USP Apparatus 2 and 4 can be attributed to

(1) aggregation of the microspheres in USP Apparatus 2; and (2) adherence of the microspheres to the fiber optic UV probe window when used in conjunction with USP Apparatus 2, as observed visually. It has been shown previously that PLGA microspheres tend to agglomerate in USP Apparatus 2 as a result of their relative hydrophobicity. The glass transition temperature (T_g) of these PLGA microspheres is 43 °C, which is close to the temperature used for investigation of in vitro release (37 °C) and consequently, the microspheres fuse together during long-term release testing in USP Apparatus 2 (Zolnik et al., 2006). This agglomeration changes the surface-to-volume ratio and hence the diffusional path length of the drug, resulting in a decrease in the release rate. Whereas, in USP Apparatus 4 the microspheres are dispersed among the glass beads in the flow-through cell thus preventing aggregation. In the initial stages of release the data obtained from USP Apparatus 2 and 4 corroborate this theory with slower release obtained with Apparatus 2 compared to Apparatus 4. At later time points, the release rate obtained using USP Apparatus 2 increased and this is considered to be due to PLGA degradation, which increases porosity and therefore surface area which counters the effects of microsphere aggregation and allows the drug to diffuse easily. Scanning electron micrographs (SEM) of these PLGA microspheres at day 20, showing degradation and increased porosity, have been previously reported (Zolnik et al., 2006).

In USP Apparatus 2, the microspheres can come into contact with the fiber optic UV probes and may adhere to the probe window. Since the microspheres are present in the dissolution vessel together with the UV probes, they may also cause light scattering. Both adherence of microspheres to the probe window and scattering from microspheres would affect the absorbance reading and may be responsible for the higher than expected cumulative release when determined using USP Apparatus 2. To avoid this problem, the probe tips were shaken prior to each reading; however, at the later time points it was difficult to remove adhered microspheres. In USP Apparatus 4 the fiber optic UV probes do not come into contact with the microspheres since they are isolated in the flow-through cells and the UV probes are contained in the release media reservoir vessels.

Release from the cefazolin microspheres did not follow the typical triphasic profile. This is considered to be a consequence of its high water solubility (350 mg/ml (Park et al., 1998)), which promotes diffusional release and loss of surface-associated drug during processing. Therefore, the lag phase that is usually associated with PLGA microspheres was not observed.

5. Conclusions

Isosbestic point monitoring provides a simple, rapid and effective means of determining cumulative drug release from MR parenterals, such as PLGA microspheres, where drug degradation occurs during release testing. This method allows detection of a drug without change in absorbance as a result of degradation and is particularly useful when investigating long-term release of rapidly degrading drugs, such as cefazolin. Isosbestic point monitoring is accurate, easy and does not require any additional steps and calculations, as are required by other methods that have been used to correct for drug degradation.

The USP Apparatus 4 appears to be a more appropriate method for monitoring cumulative drug release from PLGA microspheres when compared to USP Apparatus 2, especially when used in conjunction with fiber optic probe monitoring. This is due to the fact that the microspheres (or other dispersed systems) are isolated from the fiber optic UV probes in USP Apparatus 4 and therefore do not interfere with UV analysis. The USP Apparatus 4 method, with our modified cells containing glass beads, prevents aggregation of PLGA microspheres and consequently avoids changes in surface to volume ratios, which would affect cumulative drug release. The in situ UV spectrophotometric method is ideal for developmental work as well as for routine analysis since it is simple, rapid, and economical in terms of manpower allowing rapid evaluation of different formulations and methods. This method allows close monitoring of drug release from PLGA microspheres by adjusting sample intervals from seconds to days. This is particularly useful for systems, such as PLGA microspheres, where different release phases occur and complete information can be obtained for rapidly releasing phases.

Acknowledgements

The authors are thankful for the support of Delphian Technology, Inc. and Sotax Corporation. The awarding of a research fellowship from Pfizer, Inc. and the AFPE Gateway Research Scholarship to JMV is greatly appreciated.

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