

# Application of USP Apparatus 4 and In Situ Fiber Optic Analysis to Microsphere Release Testing

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## Abstract

The objective of this study was to determine the applicability of USP Apparatus 4 and *in situ* fiber optic UV analysis to *in vitro* release testing of poly (lactic-co-glycolic acid) (PLGA) microspheres. The release of dexamethasone from microspheres prepared with both high and low Mw PLGA was evaluated. Dexamethasone release profiles obtained using USP apparatus 4 were compared with those obtained using a sample-and-separate method. Cumulative release obtained using USP Apparatus 4 was approximately 16% higher over a 30-day period when compared to that obtained using the sample-and-separate method. This difference was attributed to loss of microspheres during separation as well as to microsphere aggregation in the sample-and-separate method. The versatility of USP Apparatus 4 with respect to alteration of flow rate allowed demonstration of diffusion-controlled release from low Mw PLGA microspheres. Fiber optic UV probes used in conjunction with USP Apparatus 4 enabled complete characterization of the initial burst release of drug from the microsphere systems.

## Introduction

In the past 15 years, the number of novel drug dosage forms such as controlled release (CR) parenterals (microspheres, liposomes, stents, and other implants) entering to the U.S. market has increased (1). These products can deliver drugs at a desired rate over periods of several days to months. It is essential to develop discriminatory *in vitro* release testing methods and standards for these delivery systems. *In vitro* release methods are necessary to monitor batch-to-batch variability, evaluate any process or manufacturing change, ensure the clinical performance of the drug i.e. safety and efficacy and determine a relationship between the *in vitro* and *in vivo* release data.

Standard dissolution methods are inappropriate for CR parenterals since: these were designed for oral and transdermal products; they utilize large volumes of media, and often require separation of the delivery system from the release media for analysis. Methods of *in vitro* release testing that are currently used for CR parenterals include membrane diffusion, sample-and-separate, *in situ* and continuous flow (2). USP apparatus 1 (basket), 2 (paddle) and 4 (flow through) have been used for microspheres and other dispersed systems. Sample-and-separate methods have been used where samples are taken from USP Apparatus 2 and the dispersed phase is isolated by centrifugation or filtration. In addition to the official USP Apparatus, other methods such as miniaturized methods and dialysis sacs have been used for CR dispersed systems. Since most of these products are small volume parenterals, miniaturized methods have been designed where the samples are placed into small vials instead of one-liter vessels. Problems with this method include violation of sink conditions, sample aggregation due to limited agitation, and disruption of the delivery system during centrifugation or filtration (e.g. coalescence of emulsion droplets, or aggregation of lipo-

some) in sample-and-separate methods leading to inaccuracy in the release data.

A recent AAPS-EUFEPS workshop report (2003) on "Assuring Quality and Performance of Sustained and Controlled Release Parenterals" stated that there is a need for standards for *in vitro* release testing methods for controlled release parenterals with respect to apparatus, media, sampling interval, and temperature, and also acknowledged that guidance on *in vivo* release testing of these products and development of *in vitro-in vivo* relationships/prediction is necessary (1).

*In vitro* release testing methods for poly (lactic-co-glycolic acid) (PLGA) microspheres are discussed herein. The 2003 AAPS-EUFEPS workshop report recommended the use of USP Apparatus 4 for *in vitro* release testing of CR microspheres (1). USP Apparatus 4, which is a flow through method that includes a pump, flow-through cells, water bath and media reservoir, was designed as an *in vitro* dissolution method for controlled release oral powders, granules, and solid dispersions. The specifications with respect to cell size of this apparatus are described in the US Pharmacopeia (3). USP Apparatus 4 can be operated under different conditions such as open or closed system mode; different flow rates and temperatures. The diversity of available cell types allows application to a wide range of dosage forms. Since the reservoir volume is not fixed, media volume can be decreased to accommodate systems where the concentration of drug released would otherwise be below the detection limit (e.g. systems with low drug loading) or increased to allow ease of maintenance of sink conditions for poorly soluble compounds. USP Apparatus 4 also offers flexibility in monitoring release via *in situ* UV analysis. Such analysis can be achieved without any correction for scattering by the dispersed system or particle accumulation on the probes since the microspheres or other delivery system are isolated

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from the media within the flow through cell. The use of *in situ* fiber optic probes in pharmaceutical analysis, different probe designs, and the challenges associated with this technology have been reviewed in the literature (4). Other reports describe fiber optic methodology with respect to linearity, precision, applicability for immediate and controlled release formulations (5), (6).

The suitability of USP Apparatus 4 as an *in vitro* release testing method for PLGA microspheres loaded with dexamethasone is described. *In situ* monitoring of drug release from microspheres using fiber optic UV probes in conjunction with USP Apparatus 4 is reported and compared with data obtained via HPLC analysis.

## Experimental Methods

### Preparation of Dexamethasone Microspheres

An oil-in-water (o/w) emulsion/solvent evaporation technique was used for dexamethasone microsphere formulation. PLGA was dissolved in methylene chloride (Fisher Scientific, Pittsburgh, PA), and dexamethasone (Sigma, St. Louis, MO) was dispersed in this solution using a homogenizer at 10,000 rpm for 1 minute. This organic phase was added slowly to 40 mL of 1% (w/v) aqueous poly (vinyl alcohol) (PVA) (Av. Mol. Wt 30,000 – 70,000 Sigma, St. Louis, MO) solution and homogenized at 10,000 rpm for 3 minutes. This emulsion was added to 500 mL of 0.1% (w/v) aqueous PVA solution and stirred at 250 rpm under reduced pressure for 6 hours. The resulting microspheres were filtered (Dura-pore Membrane Filter, 0.45 mm, Fisher Scientific, Pittsburgh, PA) washed three times and vacuum dried for 24 hours.

### Characterization of Microspheres

#### Drug Loading

10 mg of microspheres were dissolved in 10 mL of tetrahydrofuran (THF) (Optima, Fisher Scientific, Pittsburgh, PA), filtered (Millex-HV, 0.45 mm, Fisher Scientific, Pittsburgh, PA) and analyzed by HPLC method as described above for dexamethasone content.

#### Particle Sizing

An Acusizer (optical particle sizer model 770, Santa Barbara, CA, USA) was used to determine the mean particle diameter and distribution. Microspheres were suspended in 0.1% (w/v) PVA solution in water. A 500- $\mu$ l portion of this suspension was diluted with 25 mL deionized water prior to detection. All the measurements were conducted in triplicate and the mean and standard deviations are reported.

#### High Performance Liquid Chromatography (HPLC)

The concentration of dexamethasone was determined using HPLC. The HPLC system consisted of a Constametric 4100 pump (Thermoseparation), an automatic sample injector (Bio-Rad) and a UV absorbance detector (Bio-Rad) set at 242 nm. The mobile phase consisted of acetonitrile:water:phosphoric acid (30:70:0.5 v/v/v). The analytical column was Nova-Pak<sup>®</sup> C<sub>18</sub> (9 mm X 150 mm) (Millipore Corp, Waters, Milford, MA). The chromatogram was analyzed by PeakSimple Chromatography System (Model 203, software 3.29, SRI instruments, Torrance, CA) (7).

#### In Situ Fiber Optic UV Monitoring:

Type IIA UV Fiber Optic Probe dissolution system (Rainbow Dynamic Dissolution Monitor, Delphian Technology Inc., Woburn, MA) consisted of a deuterium lamp and six photo diode array spectrometers with six 0.2-cm removable path length dip type probes (Hellma Cells, Forrest Hills, NY). Indigo™ software package was used and data points were collected every 10 minutes.

### In Vitro Release Study

#### Sample-and-Separate Method

40 mg of microspheres were dispersed in 250 mL of PBS (pH 7.4) with 0.1% sodium azide in 250 mL vessels and placed in a shaker water bath (C76, New Brunswick Scientific, Edison, NJ) at 100 rpm at 37°C. At the following intervals (1, 3, 5, 7, 11 hrs and 1, 3, 5, 8, 13, 19, 23, 30 days) 1.3 mL samples were filtered to separate the microspheres from the media, replenished with 1.3 mL of fresh media and analyzed by HPLC, as described above. When the drug concentration reached 5% (w/v) of the solubility of dexamethasone, half of the total media volume was replenished. This media replenishment was taken into account in the calculation of percent release. All the measurements were conducted in triplicate and the mean and standard deviations are reported.

#### USP Apparatus 4

*In vitro* release studies were conducted using USP Apparatus 4 (Sotax CE7 smart, and CY 7 piston pump, Sotax, Horsham, PA) with flow through cells (12-mm diameter) packed with glass beads (1 mm) in a closed system mode at 37°C. 45 mg of microspheres were dispersed in the flow through cells and 250 mL of (PBS) with 0.1% sodium azide was circulated through a fiberglass filter (0.45 mm) at a flow rate of (4-35 mL/min). Samples of 1.3 mL were withdrawn, at the following intervals (1, 3, 5, 7, 11 hrs and 1, 3, 5, 8, 13, 19, 23, 27, 30 days) and analyzed by HPLC (as explained above) and replenished with 1.3 mL of fresh media. When the drug concentration reached 5% (w/v) of the solubility of dexamethasone, half of the total media volume was replenished. This media replenishment was taken into account in the calculation of percent release. All the measurements were conducted in triplicate and the mean and standard deviations are reported.

### Results and Discussion

#### Design of Apparatus 4 for Application to Microsphere Release Testing

To avoid aggregation of microspheres in the flow-through cells, the microspheres were mixed with 1-mm glass beads in the 12-mm cells. This modification of the flow-through cell is illustrated in Figure 1. The glass beads also provided laminar flow and decreased the dead volume within the flow through cells. It was determined that the ratio of microspheres to glass beads should not be too high, or back pressure problems might result. Therefore, 45 mg of PLGA microspheres (number weight average particle size 18  $\mu$ m  $\pm$

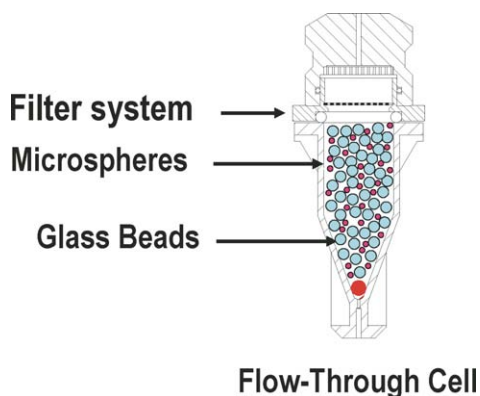


Figure 1: Schematic diagram of 12 mm flow-through cell containing microspheres and glass beads.

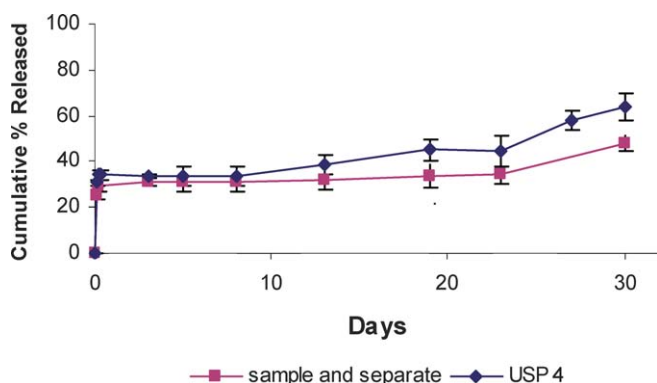


Figure 2: Dexamethasone release from PLGA (Mw 27,000) microspheres at 37°C in PBS buffer (pH 7.4) and at: (1) 20 ml/min with USP 4, and (2) 100 rpm with sample-and-separate method.

1.88) and 9 g of glass beads were selected for use in 12-mm cells. Back-pressure problems may arise due to inappropriate selection of filters. A fiberglass filter (0.45- $\mu$ m) was selected for the PLGA microspheres systems reported here.

### Comparison of USP Apparatus 4 and Sample-and-Separate Methods

PLGA microsphere systems tend to exhibit a triphasic release profile with an initial burst release of surface associated drug, followed by a lag phase where polymer degradation occurs; when polymer erosion is sufficient a second burst release of encapsulated drug occurs (8). Two different *in vitro* release methods (sample-and-separate and USP Apparatus 4) were investigated to evaluate dexamethasone release from the PLGA microspheres. The initial burst release and the lag phase up to day 7 were similar using the USP Apparatus 4 and sample-and-separate methods (Figure 2). However, after this point the release profiles started to diverge from each other resulting in a cumulative release that was 16 % higher at day 30 with USP Apparatus 4. These differences in the release profiles were considered to be due to sample loss during the filtration step of the sample-and-separate method, as well as microsphere aggregation that occurred with this method. Aggregation was evident upon

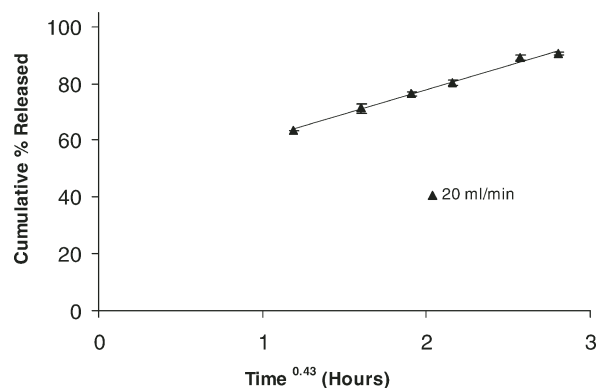


Figure 3a: Dexamethasone release from PLGA (Mw 5,000) microspheres plotted versus time to the 0.43 power (37°C, PBS buffer pH 7.4 and at 20 ml/min using USP 4).

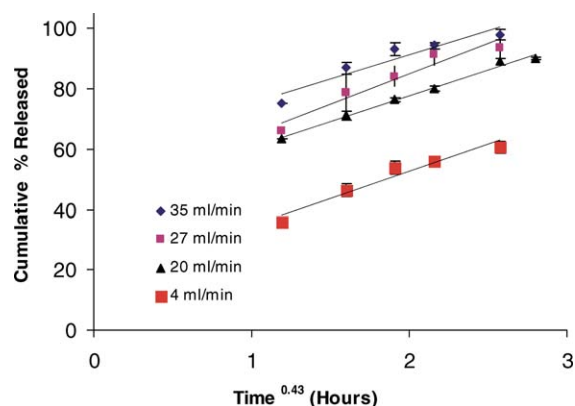


Figure 3b: Dexamethasone release from PLGA (Mw 5,000) microspheres plotted versus time to the 0.43 power (37°C, PBS buffer pH 7.4 and at 4-35 ml/min using USP 4).

visual observation of samples subjected to sample-and-separate method. In order to assure that differences between the flow rate and stirring speed used in these two methods did not contribute to the difference in the release profiles obtained, the flow rate of the USP apparatus 4 was varied between 4 and 35 mL/min. The resulting release profiles of this formulation were superimposable for all flow conditions investigated over a period of 10 days indicating that drug release was not dependent on the agitation rate for this erosion controlled high Mw PLGA microsphere system.

Microspheres prepared with low Mw PLGA exhibited rapid release and a linear relationship was established between % release and time to the 0.43 power indicating Fickian diffusion kinetics (9) (Figure 3a). In this case, increase in flow rate from 4 mL/min to 35 mL/min resulted in an increase in drug release again indicating diffusion controlled release (Figure 3b).

### In Situ Fiber Optic UV Monitoring in Conjunction with USP Apparatus 4

The use of fiber optic UV monitoring with USP Apparatus 4 offered significant advantages in investigating the initial burst release from microspheres since multiple data point

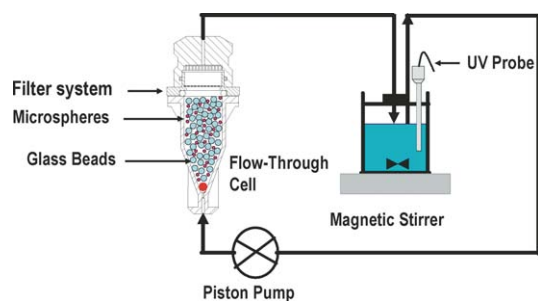


Figure 4: Schematic diagram of USP apparatus 4 showing the placement of the fiber optic probe in the reservoir vessel.

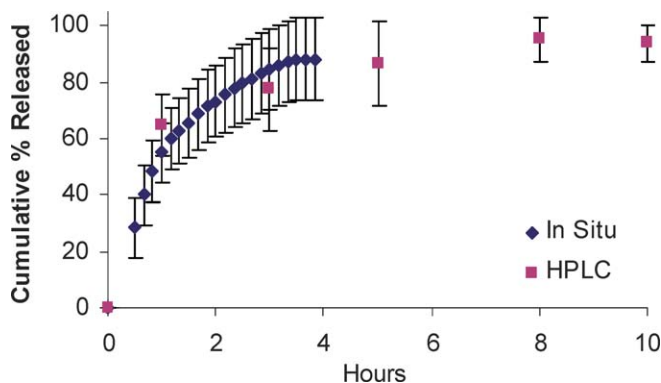


Figure 5: Comparison of *in situ* UV monitoring using fiber optic probes with HPLC analysis of the burst phase of dexamethasone release from PLGA ( $M_w$  5,000) microspheres using USP apparatus 4 (flow rate 20 ml/min, pH 7.4 and at 37°C).

collection over a short period allowed a comprehensive characterization of the burst release. This is especially important where the burst release of drug is of clinical significance or could be within the toxic range. Figure 4 is a schematic illustrating the placement of the fiber optic probe in the reservoir vessel of USP Apparatus 4 in the closed system mode. This arrangement prevents possible problems associated with particulate interference with UV analysis. Figure 5 compares *in situ* UV monitoring using fiber optic probes with HPLC analysis of the burst phase of dexamethasone release from PLGA microspheres and illustrates the utility of fiber optic monitoring to fully characterize the burst phase.

### Conclusions

USP Apparatus 4 appears to be a more appropriate method for *in vitro* release testing of PLGA microsphere systems when compared to the sample-and-separate method. The sample-and-separate method can result in aggregation problems as well as loss of sample during separation. This finding is in agreement with the recent AAPS-EUFEPS workshop (1). Fiber optic UV probes can be used in conjunction with USP apparatus 4 to monitor the release from dispersed systems, such as microspheres, since the

dispersed system is in an isolated chamber (flow through cell) and therefore does not interfere with UV analysis. The fiber optic probes allow ease of collection of multiple data points and therefore can be useful to achieve a comprehensive characterization of the release profile.

### Acknowledgments

The authors are thankful for the support of FDA, CPPR-NSF and Delphian Technology, Inc. The awarding of USP fellowship to BSZ is greatly appreciated.

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