Pharmaceutical Research, Vol. 23, No. 3, March 2006 (© 2006)
DOI: 10.1007/s11095-005-9397-8

Expert Review

Methods to Assess *in Vitro* Drug Release from Injectable Polymeric Particulate Systems

Susan S. D'Souza[1] and Patrick P. DeLuca[2,3]

Received July 19, 2005; accepted November 3, 2005

Abstract. This review provides a compilation of the methods used to study real-time (37°C) drug release from parenteral microparticulate drug delivery systems administered via the subcutaneous or intramuscular route. Current methods fall into three broad categories, viz., sample and separate, flow-through cell, and dialysis techniques. The principle of the specific method employed along with the advantages and disadvantages are described. With the "sample and separate" technique, drug-loaded microparticles are introduced into a vessel, and release is monitored over time by analysis of supernatant or drug remaining in the microspheres. In the "flow-through cell" technique, media is continuously circulated through a column containing drug-loaded microparticles followed by analysis of the eluent. The "dialysis" method achieves a physical separation of the drug-loaded microparticles from the release media by use of a membrane, which allows for sampling without interference of the microspheres. With all these methods, the setup and sampling techniques seem to influence *in vitro* release; the results are discussed in detail, and criteria to aid in selection of a method are stated. Attempts to establish *in vitro–in vivo* correlation for these injectable dosage forms are also discussed. It would be prudent to have an *in vitro* test method for microparticles that satisfies compendial and regulatory requirements, is user friendly, robust, and reproducible, and can be used for quality-control purposes at real-time and elevated temperatures.

KEY WORDS: continuous flow; dialysis; *in vitro* release methods; IVIVC; microspheres; sample and separate; subcutaneous and intramuscular administration.

BACKGROUND

Biodegradable microparticles formulated using biodegradable polymers, such as polylactide (PLA), poly(lactide-*co*-glycolide) (PLGA) (1–6), gelatin (7–9), and albumin (10,11), have been used as carriers for small molecules and biologically active peptides and proteins. In addition to being biodegradable, other advantages include reduced frequency of administration, enhanced patient compliance, sustained drug release, reduced dosage, and less systemic side effects (12,13). PLA and PLGA have been approved for human use by the United States Food and Drug Administration (US FDA) as surgical sutures, implantable devices, and drug delivery systems (14,15).

Table I lists examples of currently marketed formulations of PLA and PLGA microparticles (16). Once injected intramuscularly or subcutaneously, these formulations release the encapsulated therapeutic agent over long periods of time depending on polymer molecular weight and hydrophilicity, drug loading, and particle size and morphology (17–19). Because these formulations release drug over long periods of time, evaluation of release from microparticles requires long-term studies, *in vivo* in animals or *in vitro* using physiologic buffers at 37°C. Because of the labor and expense involved with assessing *in vivo* drug release, *in vitro* drug release studies at 37°C (physiological temperature) have gained increasing importance (20).

In vitro release studies are generally performed to accomplish one or more of the following aims (21,22):

1. As an indirect measurement of drug availability, especially in preliminary stages of product development
2. Quality control to support batch release and to comply with specifications of batches proven to be clinically and biologically effective
3. Assess formulation factors and manufacturing methods that are likely to influence bioavailability
4. Substantiation of label claim of the product
5. As a compendial requirement

An *in vitro* release profile reveals fundamental information on the structure (e.g., porosity) and behavior of the formulation on a molecular level, possible interactions between drug and polymer, and their influence on the rate and mechanism of drug release and model release data (23). Such information facilitates a scientific and predictive approach to the design and development of sustained delivery systems with desirable properties. However, in the case of parenteral drug delivery, a sizable amount of research has

[1] University of Kentucky College of Pharmacy, Lexington, Kentucky 40536, USA.
[2] Faculty of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, Kentucky 40536, USA.
[3] To whom correspondence should be addressed. (e-mail: ppdelu1@uky.edu)

Table I. List of Currently Marketed Preparations Formulated Using PLA and PLGA

Product	Polymer	API	Company
Lupron Depot®	PLA or PLGA	Leuprolide	TAP
Trelstar™ Depot	PLGA	Triptorelin	Pfizer
Decapeptyl® SR	PLA or PLGA	Triptorelin	Ipsen-Beaufour
Decapeptyl®	PLGA	Triptorelin	Ferring
Suprecur® MP	PLGA	Buserelin	Aventis
Nutropin®	PLGA	Human growth hormone	Genentech
Sandostatin® LAR	PLGA-glucose	Octreotide	Novartis
Somatuline® LA	PLGA	Lanreotide	Ipsen-Beaufour
Arestin®	PLGA	Minocycline	OraPharma
Risperdal Consta®	PLGA	Risperidone	Janssen Pharmaceutica

PLA: polylactide; PLGA: poly(lactide-*co*-glycolide); API: active pharmaceutical ingredient

focused on biodegradable microparticles as efficacious delivery systems, but very little attention has been devoted to the development of an *in vitro* release technique.

Unlike controlled release oral formulations, there are no regulatory standards for parenteral microparticle delivery systems. Also, the current United States Pharmacopeia (USP) apparatus (24) for *in vitro* release testing was designed mainly for oral and transdermal products and is not directly applicable for parenteral products administered subcutaneously or intramuscularly. For example, concerns with using USP apparatuses 1 (basket) and 2 (paddle) include sample containment, large volume of media required for testing, and sampling procedure. USP apparatuses 5 (paddle over disc), 6 (cylinder), and 7 (reciprocating holder) were designed for the transdermal route and do not offer any advantages for parenteral delivery systems such as microparticles. Additionally, drawbacks of USP apparatuses 3 (reciprocating cylinder) and 4 (flow-through cell), designed for extended-release oral dosage forms, include evaporation (reciprocating cylinder) and filter blockage along with polymer migration leading to variable flow rates (flow-through cell).

Over the past decade, there have been attempts to compare *in vitro* test methods to study drug release from parenteral biodegradable microspheres. Nastruzzi *et al.* (25) studied the release of bromocriptine mesylate from Parlodel LA® using dialysis tubes and a flow-through cell method and also compared the reproducibility between the two *in vitro* tests. In the dialysis method, a dialysis tube containing 20- to 25-mg microspheres was placed into 100-ml of 50 mM citrate buffer pH 3.5, whereas in the flow-through cell method, 20-mg microspheres were packed in a column (45 × 9 mm) filled with 3 ml of 50 mM citrate buffer pH 3.5 with a flow rate of 0.12 ml/min. Very different release rates were obtained, although the overall shapes were similar (Fig. 1). Greater release of drug with a longer time to plateau occurred with the dialysis technique, whereas with the flow-through cell, the time to reach the plateau was shorter, but the amount released was smaller. Conti *et al.* (26) assessed release from indomethacin PLA microspheres using the USP dissolution test apparatus, rotating bottle apparatus, shaker incubator, and a recycling flow-through cell. The following test conditions were employed to assess *in vitro* release in buffer:

1. USP XII paddle dissolution test apparatus: 1000-ml buffer at 100 and 200 rpm
2. Rotating bottle apparatus: 100-ml buffer at 29 rpm
3. Shaker incubator: 100-ml buffer at 60 and 120 strokes/min
4. Recycling flow-through cell: 1000-ml buffer at a flow rate of 17 and 33 ml/min

For the indomethacin PLA microspheres, drug release was fastest with the recycling flow-through cell with similar release profiles obtained using the USP dissolution XXII apparatus, shaker incubator, and rotating bottle apparatus (Fig. 2). Results from experiments with bromocriptine and indomethacin microspheres were similar in that *in vitro*

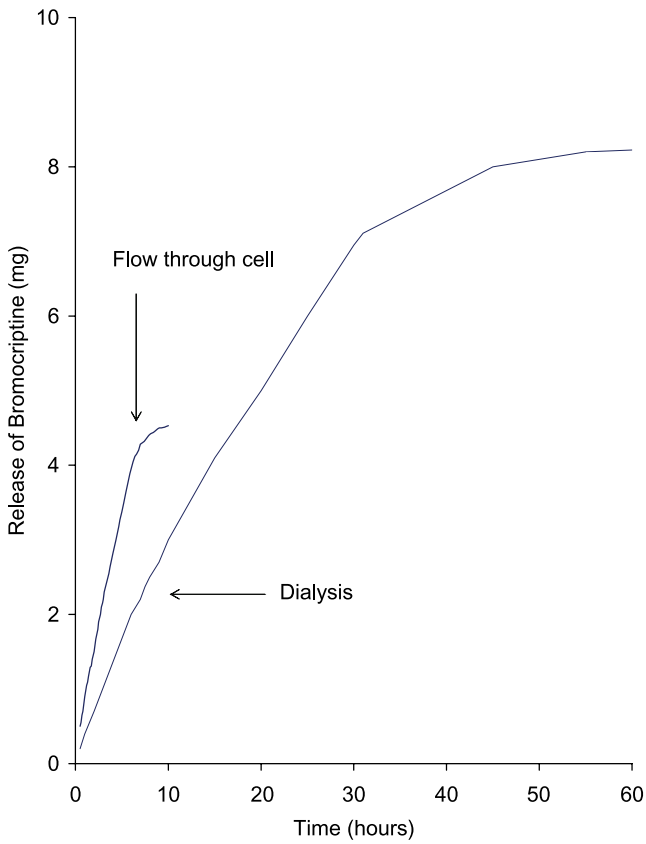

Fig. 1. Release of bromocriptine from poly(lactide-*co*-glycolide) (PLGA) microspheres determined using dialysis and flow through cell [redrawn from Nastruzzi *et al.* (25)]. Note that the studies were continued until a plateau was reached.

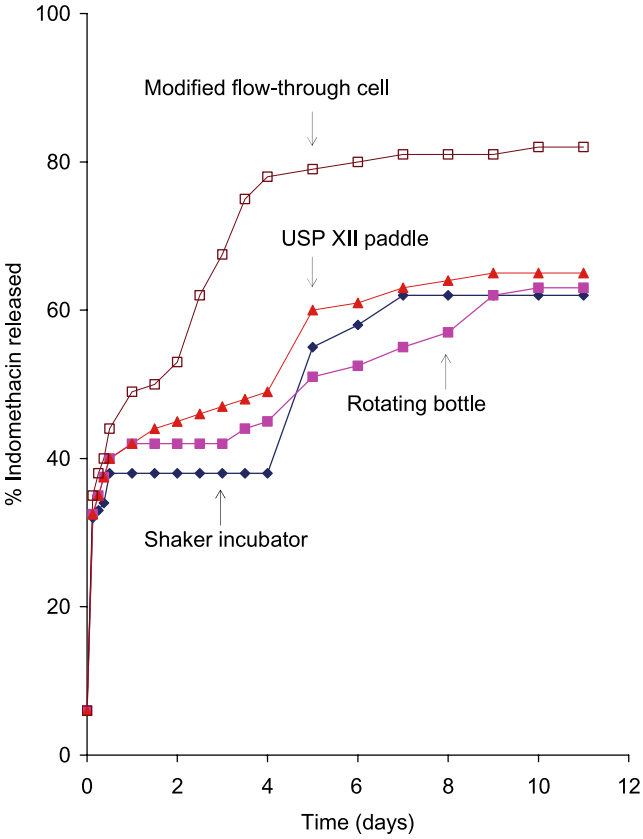

Fig. 2. Release of indomethacin PLGA microspheres assessed using USP XXII Paddle, rotating bottle, shaker incubator, and modified flow-through cell methods [redrawn from Conti *et al.* (26)].

release with the flow-through cell was faster. *In vitro* dissolution tests on depot microsphere formulations of doxepin using a membrane and a nonmembrane system (Fig. 3) by Gido *et al.* (27) revealed slower release rates and incomplete drug release with the membrane system. Studies on *in vitro* release of spray-dried rifampicin microspheres formulated using a blend of R104 polymer (D,L-PLA, MW 2000) with R202H (D,L-PLA, MW 9000) were performed using bottles shaken horizontally in a water bath and with the USP dissolution apparatus (28). Briefly, about 10 mg of microspheres was added to a bottle containing 100-ml dissolution medium with horizontal shaking at 1.5 Hz, whereas in the USP XXII test method, release of 50-mg microspheres was assessed in 500-ml dissolution medium agitated at 100 rpm at 37°C. Figure 4 shows that drug release was significantly faster with the USP paddle apparatus. Because the ratio of microspheres to dissolution media was the same, faster release with the USP paddle apparatus was attributed to the greater degree of agitation with the paddle, which prevented the microspheres from forming aggregates at the base of the vessel. When agitation was employed, the microspheres remained as individual particles and were continually suspended in the media, resulting in faster release.

In recognition of the need for a standard *in vitro* release method, a series of national and international workshops on quality assurance and performance of sustained and controlled release parenterals have been conducted in recent years (29–32). The issues addressed in addition to methodology were apparatus, outcomes, parameters necessary for method development, and *in vitro–in vivo* correlation (IVIVC) for sustained release parenteral dosage forms. The resulting publications included important guidelines for "novel" or "special" dosage forms, including implants, injectable microparticle formulations, and liposomes (31). These delivery systems were categorized as those "dosage forms requiring more work before a method can be recommended." Some of the concerns in need of resolution included evaporation, prevention of microbial contamination, osmolarity, pH, and buffer capacity of the media for these extended release formulations, as the time to conduct *in vitro* studies would encompass weeks or months.

Currently, research is focused on shortening the time span of *in vitro* release experiments with the aim of providing a quick and reliable method for assessing and predicting drug release (33,34). For commercial dosage forms that release drug for 30–90 days or even longer, accelerated or short-term release provides the potential for conducting an *in vitro* release test in a matter of days rather than months. Release testing of these dosage forms at 37°C would require the addition of preservatives and impose certain limitations on the *in vitro* method, such as stability and compatibility of the components of the release device, like tubings and membranes. Therefore, a short-term release test might even be more reliable for quality-control purposes. In addition, short-term studies can provide a rapid assessment of formulation and processing variables that affect drug release from the delivery system, especially in the developmental stages. These short-term studies can be performed by accelerating

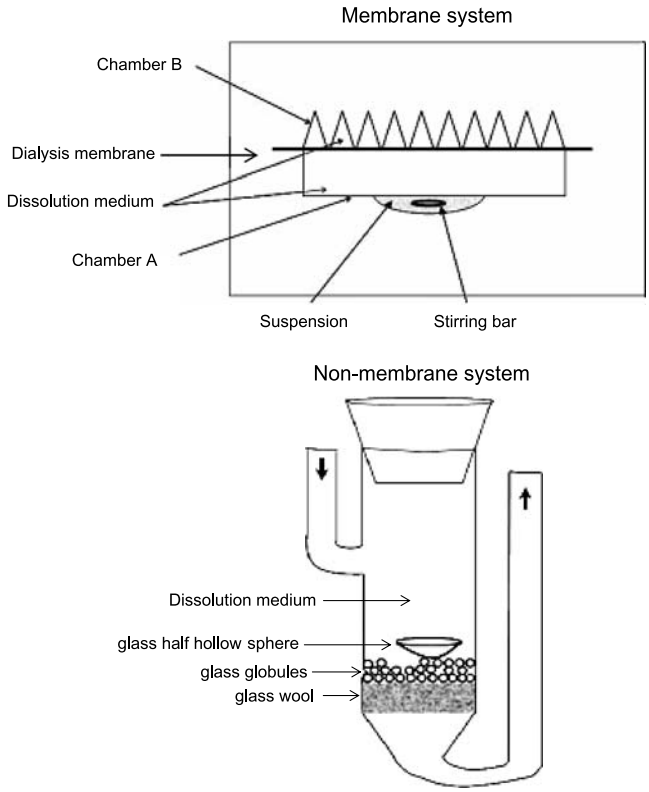

Fig. 3. Cross-sectional views of the membrane and non-membrane system used to assess release of Doxepin from parenteral depot formulations [redrawn from Gido *et al.* (27)].

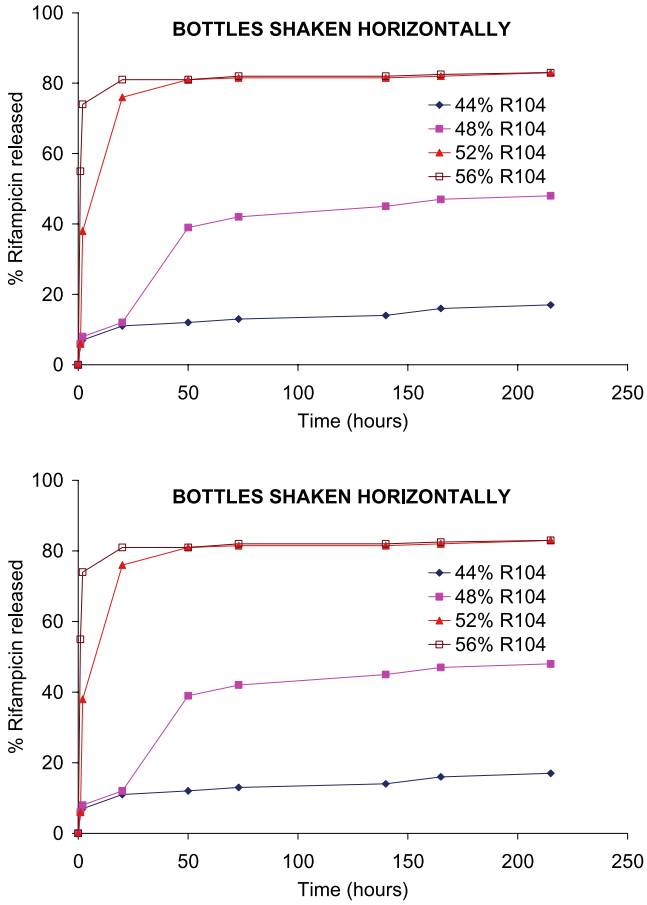

Fig. 4. *In vitro* release of rifampicin from physical blends of R104 polymer (D,L-PLA, MW 2000) microspheres with R202H (D,L-PLA, MW 9000) using two methods [redrawn from Bain *et al.* (28)].

one or more conditions employed in a real-time *in vitro* release study. Such accelerating conditions include elevated temperature, altering pH, and use of surfactants. As with the real-time *in vitro* release study, the method should be simple, reproducible under the conditions of study, inexpensive, and applicable to biodegradable microsphere formulations that have varying duration of action *in vivo*.

The aim of this review is to provide the reader with a brief description of the currently used *in vitro* release methods for particulate systems along with their advantages and disadvantages. It is hoped that such a compilation would aid the selection of an appropriate release method that could be used, first, as a quality-control tool in commercial and clinical product batch release and, second, as a research tool during formulation development. The ultimate criterion for acceptance of an *in vitro* release method is having an IVIVC. This compilation of the various methods should aid the regulatory agency (FDA) in soliciting information on commercial batches that permit IVIVC.

CONSIDERATIONS IN METHOD DEVELOPMENT

As with conventional release testing, selection of media and temperature are important. Media selection is governed by drug solubility and stability over the duration of the study, whereas the temperatures employed may be physiological, 37°C, or elevated (33,34). Additionally, the following should be considered prior to studying drug release:

1. *Sink conditions:* Although sink conditions may not exist at the *in vivo* site of injection, it is wise to employ sink conditions during *in vitro* testing. In the event that a small volume of media can be used (based on the method employed and assay sensitivity), total media replacement may be used to ensure drug solubility, maintain sink conditions, and prevent accumulation of polymer degradation products.
2. *Burst release:* The release method employed should be able to identify a high initial release or "burst" from the formulation. Additionally, the method should provide information about the onset and duration of burst to assess its influence on the *in vivo* efficacy and safety window of the drug being studied.
3. *Robustness of technique:* The *in vitro* release method employed should be able to assess the influence of changes in the manufacturing procedure on the formulation. This would be useful from a quality-control standpoint and could also aid in the design and development of microparticle drug delivery systems.

ADDITIONAL CONSIDERATIONS

Currently, *in vitro* release testing of controlled and sustained release parenterals is primarily for quality-control purposes. However, as with controlled release oral and transdermal formulations, the outcome of *in vitro* release tests should be to ensure clinical performance, i.e., safety and efficacy of the product. To achieve *in vivo* relevance, physiological variables at the site need to be considered including body temperature and metabolism (factors that affect blood flow), muscle pH, buffer capacity, vascularity, level of exercise, and the volume and osmolarity of the product. Other considerations include tissue response (inflammation and/or fibrous encapsulation of the product). The lack of such information has prompted the FDA to exercise caution in establishing regulatory guidelines.

Some guidelines that have been suggested for *in vitro* method development include (29):

1. Identification and selection of release media and conditions that result in reproducible drug release rates
2. Preparation of formulation variants that are expected to have different behavior *in vivo*
3. *In vitro* and *in vivo* release testing of formulation variations
4. Modification and/or selection of an *in vitro* release method that discriminates between formulation variants that exhibit different *in vivo* release profiles

Ideally, an *in vitro* test method should mimic *in vivo* conditions and release mechanism as much as possible.

DESCRIPTION OF *IN VITRO* RELEASE METHODS

The most commonly used methods for microparticulate systems can be grouped into three broad categories, viz., sample and separate methods (SS), continuous flow (CF),

and dialysis (D). A brief description of each of these methods will be provided along with references, advantages, and disadvantages.

Sample and Separate Methods

This is the most widely used technique (13,35–74). Briefly, drug-loaded microparticles are introduced into a vessel containing media and release is assessed over time. Media selection is based on drug solubility and stability over the duration of the release study. Modifications of the basic technique to study drug release include size of container, use of agitation, and sampling methods.

Container size. Container selection depends on the volume of dissolution media necessary to maintain sink conditions without compromising the sensitivity of the assay for the activity being studied. For example, *in vitro* release studies have been performed in tubes or vials when small volumes (<10 ml) are used (49,53,65) and bottles or Erlenmeyer flasks when larger volumes (100–400 ml) (38,48) of media are required.

Type, extent, and use of agitation. Once suspended in media, microparticles may be subjected to continuous or intermittent agitation for the duration of the release study. Agitation of microspheres using a paddle was reported to prevent aggregation of microspheres, which significantly reduced the release rate from rifampicin microspheres (28). Continuous agitation may be provided by using a magnetic stirrer at a fixed speed (74), wrist shaker rotating at 360° (44), incubator shaker (46), shaking water bath (45,47,72), tumbling end-over-end (68), or high-speed stirring/revolution of bottles (46). In some cases, the media contents were kept static during incubation at 37°C (70).

Sampling technique. Drug release is monitored at intermittent intervals by separating the particles from the bulk media either by filtration or centrifugation. Filtration of media contents is accomplished using membrane filters having a size that can filter polymer fragments followed by analysis of supernatant (38,47). Centrifugation of media contents is also widely used and may be followed by sampling of the supernatant (70,75,76) or analysis of remaining drug in the microspheres as with etoposide (35), peptides such as vapreotide, a somatostatin analog (41), leuprolide, a luteinizing hormone–releasing hormone analog (52), and thyrotropin-releasing hormone (TRH) (77), because of instability in the release media. As an alternative to filtration or centrifugation of microparticles, Bodmeier and McGinity (54) allowed the media contents to settle before sampling the supernatant. The volume of supernatant withdrawn depends on drug solubility and stability, assay sensitivity, and maintenance of sink conditions. For poorly water-soluble drugs, such as paclitaxel, all of the release media (10 ml) was withdrawn at each analysis followed by replacement with the exact volume sampled (36). A similar procedure was adopted for interferon-α where low loading (1.1%) necessitated the removal of 3-ml supernatant from the release media (4 ml) (37). For drugs such as amoxicillin, which are unstable in media, complete withdrawal of supernatant was achieved by centrifugation followed by analysis of remaining native drug in microspheres and supernatant (72).

Buffer replacement. Buffer replacement is necessary to maintain sink conditions postsampling. In some cases, total buffer replacement is necessary to prevent the accumulation of drug degradation products in solution (44). For samples subjected to filtration, buffer replacement is accomplished by "back-washing" as reported by Hickey *et al.* (13). For centrifuged samples, buffer replacement is generally followed by resuspension of microparticles (69).

Advantages and disadvantages. This technique provides a direct and reasonably accurate assessment of *in vitro* release. However, aggregation of microspheres is a major concern and may lead to lower release rates (28). To minimize effects of agitation, surfactants have been used (33,34) and/or intermittent shaking of media contents was performed (55). Sampling is another major issue, especially when filtration or centrifugation is used. Small-sized particles (<10 μm) lead to filter clogging when polymer degradation and dissolution occur. Loss in volume because of filtration during sampling and buffer replacement is a concern when the amount of release media is small. Sampling by filtration cannot be used with drugs that bind to the filter.

Centrifugation followed by analysis of the supernatant is an alternative to filtration. However, time to sediment increases as the particles start degrading. Also, redispersion of the degraded particles is difficult. Because release studies for these extended release dosage forms could run into months, total buffer replacement is sometimes necessary to maintain sink conditions. This is very difficult to accomplish if filtration or centrifugation is used as the sampling technique. Alternatively, the microparticles could be recovered at periodic intervals and remaining drug analyzed (35). This destructive technique requires a large amount of microparticles and is not an attractive option to study release.

Continuous Flow Methods

Modifications of the USP apparatus 4 have been used to assess drug release from parenteral microparticles. Briefly, media is circulated through a column containing drug-loaded microparticles, and release is assessed over a period of time. As with the SS technique, media selection is based on drug solubility and stability over the duration of the release study. A variety of setups, pumps, and flow rates have been reported in the literature and are stated below.

CF methods used. In one of the earliest setups reported, microparticles were added to a small amount of media contained in a filtration cell (78,79), the base of which was covered by a large area filter. Media was continuously pumped into the cell, and samples were removed by filtration from the base of the cell for drug analysis, often by on-line automated accessories. Fresh media was pumped into the cell to keep the volume constant. A variant of this method is continuous flow of buffer through a jacketed column (37°C) containing drug-loaded microparticles (80–86) or placed in a circulating water bath (87).

Although most of the CF methods reported are developed in-house, some studies have utilized commercially available automated equipment (88,89) Sotax AG (Basel, Switzerland). Figure 5 shows the equipment used by Wagenaar *et al.* (88) to study drug release from Piroxicam PLA and PLGA

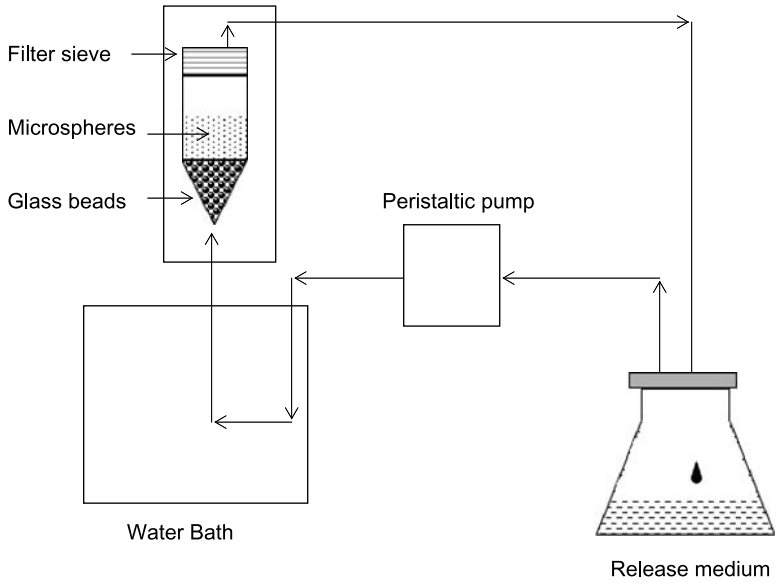

Fig. 5. Commercial apparatus for continuous flow method using peristaltic pump with recirculating buffer [adapted from Wagenaar and Muller (88)].

microspheres. A weighed amount of microspheres was placed on top of glass beads in a CF cell, and release media was recirculated through the cell.

Pumps and flow rates. Constant flow of media is achieved by using a peristaltic (83,88,89) (Figs. 5 and 6), syringe (80–86) (Fig. 7), or high-performance liquid chromatography (HPLC; Fig. 8) (87) pump. With the CF setups using peristaltic pumps, the buffer may be recirculated (88) (Fig. 5) or the fresh buffer may be pumped constantly (83) (Fig. 6) through the system. Cortesi *et al.* (83) used a peristaltic pump to achieve constant flow through the column. At the base of the column, the media was subjected to stirring using a magnetic stir bar prior to eluent collection and analysis (Fig. 6). Aubert-Pouëssel *et al.* (80) used a syringe pump (Fig. 7) connected to a jacketed column at 37°C to assess the *in vitro* release of GDNF from PLGA microspheres. Column temperature was maintained at 37°C using a circulating water bath (87) (Fig. 8). Selection of a flow rate seems to depend on the type of pump used to study *in vitro* release. Flow rates varying from 5 µl/min (80,82) have been obtained with syringe pumps, 0.4 ml/min (87) with HPLC pumps, and up to 200 l/h with peristaltic pumps (88).

Table II lists examples of parenteral microsphere formulations where *in vitro* release was assessed using the CF method. In most cases, a lower flow rate resulted in incomplete release probably because of slower rates of hydration and dissolution of the polymer and drug, respectively. Conversely, cumulative release greater than 85% was obtained with higher flow rates. Hydration of the polymer matrix is the most important factor governing the release from microparticulate delivery systems. Once the polymer is hydrated, drug release occurs as a result of a combination of diffusional and erosional processes. With the flow-through cell method, a lower flow rate will lead to slower hydration of the polymer matrix. For example, *in vitro* release of water-soluble ssDNA and dsDNA formulated using gelatin

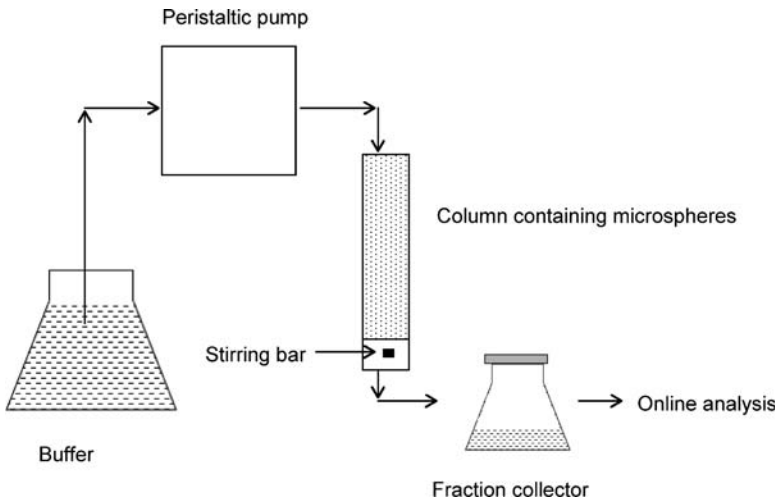

Fig. 6. Apparatus for continuous flow method using a peristaltic pump [adapted from Cortesi *et al.* (83)].

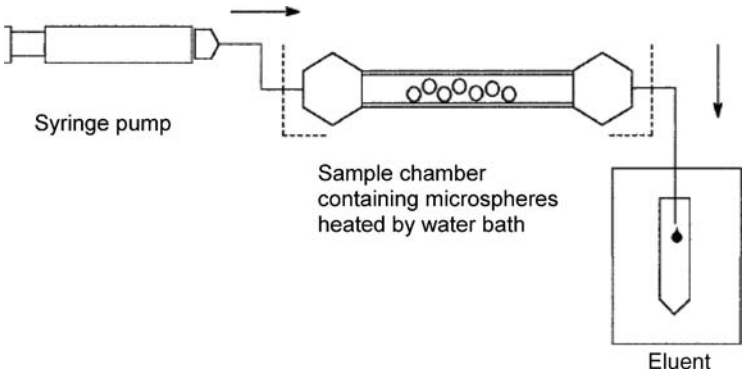

Fig. 7. Apparatus for continuous flow method using syringe pump [adapted from Aubert-Pouëssel *et al.* (80)].

(a water-soluble polymer) was found to be 70 and 30% within 250 min (4.16 h), respectively, at a flow rate of 0.12 ml/min (Table II). This difference in release is presumably a result of the differences in solubility of the DNA moieties. Likewise, an increase in the flow rate (25 ml/min) with clonidine/gelatin microspheres showed faster release (~85% in 7 h) of the water-insoluble drug. This suggests that flow rate is an important parameter in the assessment of drug release when the CF method is used. Another parameter to be considered is the volume of buffer, which depends on drug solubility and assay sensitivity. In the event that buffer is being recirculated, it is important that sink conditions be maintained by replacing part or all the buffer.

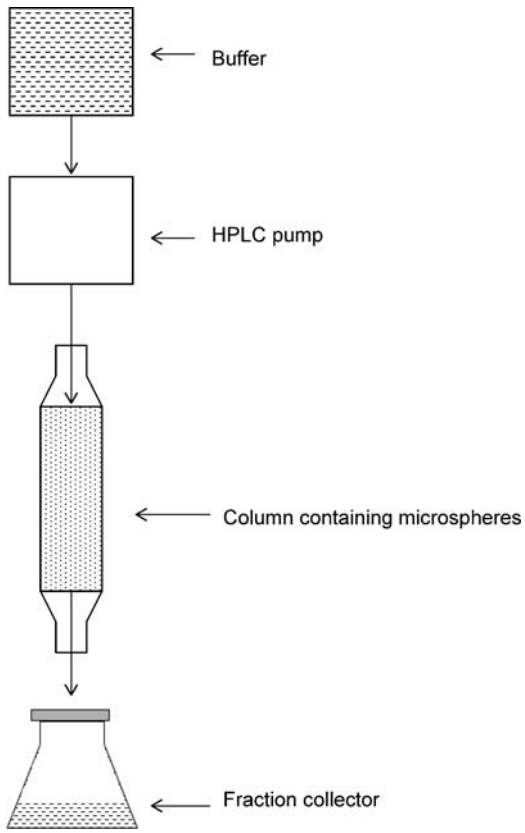

Fig. 8. Apparatus for continuous flow using high-performance liquid chromatography pump [adapted from Longo and Goldberg (87)].

Advantages and disadvantages. The CF method attempts to simulate the *in vivo* environment by constantly circulating a small volume of media through immobilized microparticles to hydrate the particles and cause dissolution and diffusion of the drug. A major advantage of this method is that samples can be continuously and conveniently sampled and analyzed along with buffer replacement because of the automated process. Disadvantages with this procedure include variation in the flow rate due to clogging of the filter (because of polymer degradation) leading to high-pressure buildup in the system. Also, low flow rates are achieved with the types of filters used (membrane and ultrafilters) and seem to be responsible for low rate and extent of drug release from microsphere formulations. Lastly, rapid replacement of the buffer is difficult to achieve in practice.

Dialysis Methods

In this method, drug-loaded microparticles are physically separated from the bulk media by a dialysis membrane, and release is generally assessed from the outer bulk over time. In some cases, instability of drug in the media necessitates the sacrificing of microspheres followed by analysis of the remaining drug (90). Originally, the dialysis technique was used to study drug release from oily parenteral depot solutions (91–93) and suppositories (94), particulate-based injectable formulations of poorly water-soluble drugs (95), and liposomes (96). More recently, this technique has been used to study drug release from a variety of particulate systems for topical preparations (97), oral suspensions (98), submicron emulsions (99), and intranasal (100) delivery. Other novel dosage forms where the dialysis technique has been used include nanoparticles (101–105), implants (106), and micelles (107). Media selection is based on drug solubility and stability over the duration of the release study. Various modifications of the basic technique have been employed to assess drug release and are described below.

Dialysis methods used. The most commonly reported setups utilize a dialysis bag (25,101–104,108–115) where a suspension of the microparticles is introduced into the bag that is sealed and placed in a vessel containing buffer. Drug diffusion from the dialysis bag into the outer sink may be increased by agitating the vessel contents, thereby minimizing unstirred water layer effects. Common modes of agitation

Table II. Examples of Release Conditions and Results with CF Methods

Drug/polymer	Flow rate (ml/min)	Method details	% Release (time)	Reference
Lysozyme/PLGA	0.005	Syringe pump	20% (8 weeks)	Aubert-Pouëssel *et al.* (80)
GDNF/PLGA	0.005	Syringe pump	60% (8 weeks)	Aubert-Pouëssel *et al.* (82)
GDNF/PLGA	0.005	–	30% (8 weeks)	Jollivet *et al.* (86)
Bromocriptine/PLGA	0.12	Continuous elution	35% (12 hrs)	Nastruzzi *et al.* (25)
ssDNA/Gelatin, dsDNA/Gelatin	0.12	Peristaltic pump	70% (250 min) 30% (250 min)	Cortesi *et al.* (83)
Adriamycin/albumin	0.4	High-performance liquid chromatography pump	90% (14 hrs)	Longo and Goldberg (87)
Indomethacin/PLGA	17 and 33	Peristaltic pump	85% (11 days)	Conti *et al.* (26)
Piroxicam/PLGA	3.33	Peristaltic pump	85% (50 hrs)	Wagenaar and Muller (88)
Clonidine/gelatin	25	Peristaltic pump	85% (7 hrs)	Vandelli *et al.* (89)

include a horizontal shaker (25,115) or using the USP paddle apparatus (112,113) under agitation. Other setups include a tube with dialyzing membrane at one end (Fig. 9) (90,116–118) or two chambers separated by a dialysis membrane (27). Membrane molecular weight cutoffs (MWCO) reported a fall in a broad range; for example, MWCOs of 3500 Da have been used for bovine serum albumin (119), 6000–8000 Da for 5-fluorouracil (115), and 300,000 Da for small peptides such as orntide (116). From the literature reports, the rationale for the selection of membrane MWCO seems arbitrary, with sufficiently high membrane MWCOs selected for *in vitro* release studies so as not to be a limiting factor for drug diffusion.

The dialysis technique has been employed with small molecules (115), peptides (108,110,116–118), and proteins (119). Volume of media inside the dialysis membrane containing the microparticles is at least 6- to 10-fold less than that of the outer bulk, providing a driving force for drug transport to the outside and maintaining sink conditions. Most studies comparing the dialysis technique with the tube method show differences in release rate and profile (110,119,120). *In vitro* release of calcitonin (MW 3600) from microspheres using both the "tube method" with agitation and "dialysis bag (MWCO 12–14 kDa)" showed complete release with both methods, with release being slower with the dialysis technique but more reproducible (110). In another report, the tube method showed slower release when compared to a dialysis bag (MWCO 12–14 kDa), which was selected to study the *in vitro* release of ^{125}I-bovine calcitonin from PLGA microspheres, as it offered more advantages over the tube method (120). *In vitro* release of two proteins, carbonic anhydrase (MW 31 kDa) and bovine serum albumin (MW 66 kDa), from PLGA microspheres from a "dialysis bag (MWCO 3.5 kDa)" was compared to the "tube method" (119). Both proteins were shown to be stable and active in the supernatant and microspheres when the dialysis method was used. It was believed that the dialysis bags permitted a constant pH because water-soluble oligomers, from polymer degradation, were removed, resulting in slower polymer degradation and greater stability of the protein. In addition, dialysis bags simulated the *in vivo* environment and retained sink conditions better than the tube method.

The findings of these studies, however, should be interpreted with caution. In the aforementioned studies, the volume used for studying release from the tube method was equal to the volume added to the dialysis bag. However, total volume of media used in the dialysis method [69 *vs.* 10 ml (110), 80 *vs.* 1 ml (120), and 2000 *vs.* 4 ml (119)] was much larger than with the tube method. The low volumes (1–10 ml) used with the tube method would not be able to provide adequate buffer capacity, leading to build up of acidic

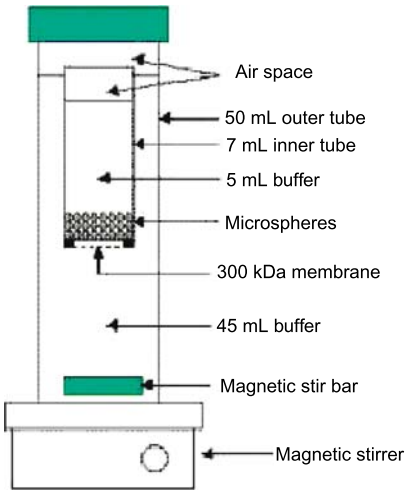

Fig. 9. Dialysis method utilizing membrane at one end [from Kostanski and DeLuca (116)].

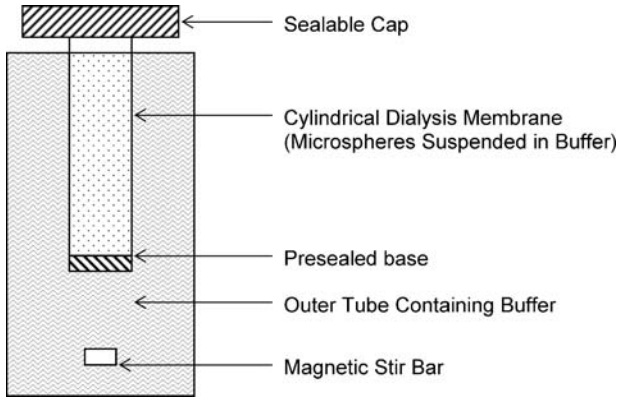

Fig. 10. Commercially available dialyzer [from D'Souza and DeLuca (122)].

Table III. Attempts at *In Vitro–In Vivo* Correlation Using SS, CF, and D Methods with Biodegradable Microparticles

Drug/PLGA	*In vitro* release conditions at 37°C	*In vivo* studies	Figure no.	Reference
Orntide	D (membrane at one end), 0.1 M AB pH 4.0	Male S–D rats	11	Kostanski *et al.* (116,117)
Lysozyme	SS (orbital rotation), 0.1 M GH buffer pH 2.5	Male S–D rats	12	Jiang *et al.* (75)
bSOD	SS (orbital rotation), 0.1 M PBS pH 7.4	Male Wistar rats	13	Morita *et al.* (124)
Vapreotide	SS (orbital rotation), fetal bovine serum albumin	Male S–D rats	14	Blanco-Prieto *et al.* (41)
TRH	SS (shaking), 0.033 M PB pH 7.0	Male Wistar rats	15	Heya *et al.* (125)
TRH	SS (orbital rotation), 0.033 M PB pH 7.0	Male Wistar rats	16	Heya *et al.* (126)
Methadone	SS (stirring), 0.066 M PB pH 7.4 + 0.001% T-80	Male Swiss mice	17	Negrin *et al.* (74)

S–D: Sprague–Dawley; SS: sample and separate methods; CF: continuous flow; D: dialysis; GH: glycine–HCl buffer; AB: acetate buffer; PB: phosphate buffer; PBS: phosphate-buffered saline.

degradation products resulting in peptide/protein instability in the outer media and in the acidic microenvironment of the microspheres.

Advantages and disadvantages. The dialysis method is attractive because sampling and media replacement are convenient because of physical separation of the microparticles from the outer media by a dialyzing membrane. Although the dialysis technique has been criticized because of its low *in vivo* predictability in the case of intravenous or oral administration of microparticles (23), it mimics *in vivo* conditions where the microparticles are immobilized upon administration (subcutaneously or intramuscularly) and surrounded by a stagnant layer causing slow diffusion of drug because sink conditions are not maintained (25). Achievement of equilibration with the outer media is slow if membrane surface area is small, i.e., membrane at one end of a tube, and would limit an accurate analysis of initial drug levels in formulations where the burst effect is high (116). However, this issue was addressed by using dialysis bags (more surface area) where initial release over 24 h was about 88% (108). Another disadvantage is that the time to equilibrate is prolonged if the bulk media is not stirred (formation of unstirred water layer). In such situations, it is recommended that the outer media be agitated to minimize unstirred water layer effects and to prevent accumulation of polymer degradation products, especially when the formulation contains a protein (119). Also, this technique cannot be used if the drug binds to the polymer or membrane (121). However, because of the ease of sampling and the possibility of total buffer replacement, this method seems to be an attractive option to study drug release from microparticles and other particulate dosage forms.

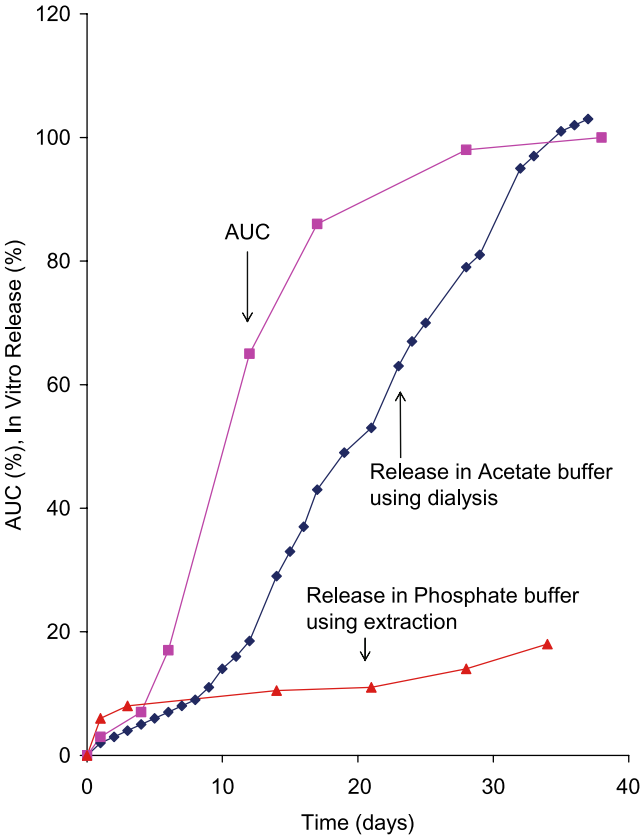

Fig. 11. Comparison of % AUC with % *in vitro* release for orntide PLGA microspheres [redrawn from Kostanski and DeLuca (116)].

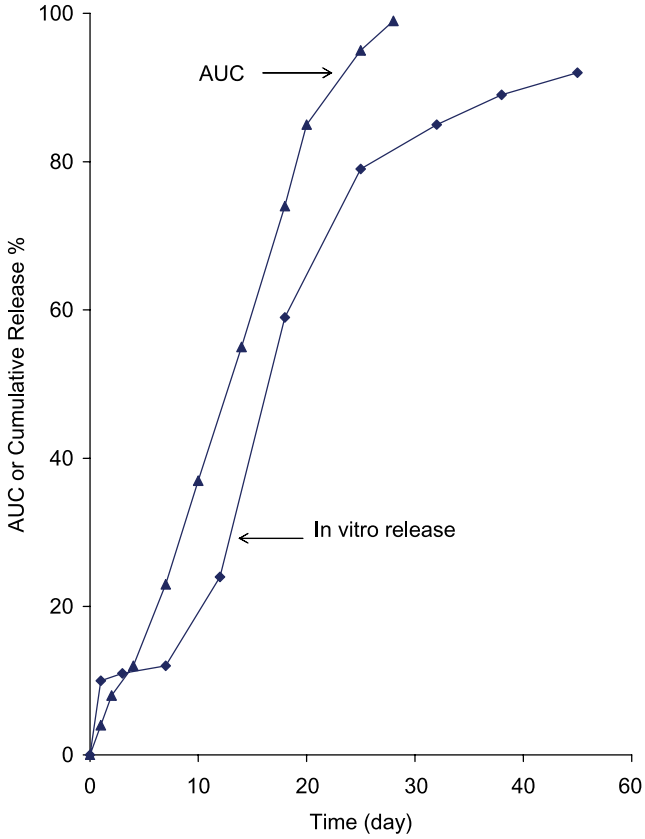

Fig. 12. *In vitro–in vivo* correlation (IVIVC) for lysozyme 50:50 PLGA (RG502H) microspheres [redrawn from Jiang *et al.* (75)].

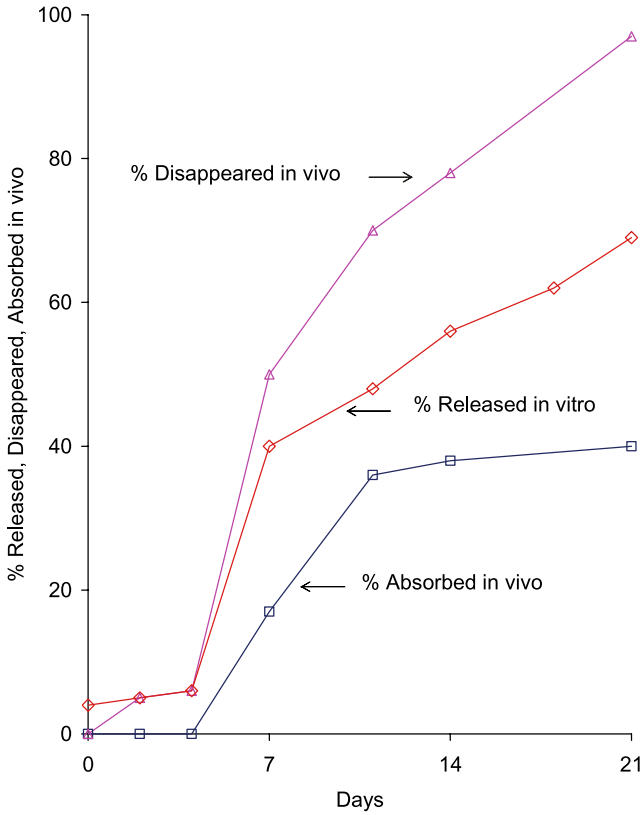

Fig. 13. IVIVC for bovine-derived superoxide dismutase PLGA microspheres [redrawn from Morita *et al.* (124)].

Disadvantages with the dialysis technique are a cumbersome setup procedure for dialysis bags (120) and membrane at one end of a tube (90). This can be addressed by using a commercially available dialyzer (122) having a large surface area (Fig. 10). Additionally, the regenerated cellulose membrane is stable up to 60°C, which would allow its use in short-term elevated temperature studies (33,34). Also, the membrane may be washed and reused after each experiment, which would render it cost effective.

IVIVC WITH PARENTERAL MICROSPHERES

Drug release from dosage forms can be directly assessed through *in vivo* bioavailability studies in humans or animals. However, the length of time needed to plan, conduct, and interpret results from an *in vivo* study, the high cost of the study, and the unnecessary use of human subjects are the reasons why investigations of IVIVC between *in vitro* dissolution and *in vivo* bioavailability are increasingly becoming an integral part in the development of extended release products (123). For this reason, *in vitro* release tests have been used as an indirect measurement of drug availability, especially in preliminary assessments of the formulation factors and manufacturing methods that are likely to influence bioavailability. Therefore, it is imperative that the results of the *in vitro* dissolution tests correlate with *in vivo* bioavailability measurements.

Several reports on IVIVC for injectable PLGA microspheres have been published in the past decade on a variety of therapeutic agents (Table III). These include proteins such as lysozyme (75), bovine-derived superoxide dismutase (124), and peptides such as orntide (116,117), vapreotide (41), TRH (125,126), and small molecules such as methadone (74). Sprague–Dawley (S–D) or Wistar species of the rat are the most commonly used models for *in vivo* experiments. With orntide microspheres, *in vivo* data (%AUC) were compared with *in vitro* release from 0.1 M acetate buffer (AB) pH 4.0 using a dialysis method and from 0.1 M phosphate buffer (PB) pH 7.4 (tube method) by extraction of peptide by recovering the microspheres (116). A better IVIVC (Fig. 11) was obtained with 0.1 M AB pH 4.0 than 0.1 M PB pH 7.0 and was ascribed to the pH-dependent solubility of the peptide, which was higher at lower pH (116). Also, *in vitro* release using the dialysis method for orntide microspheres was found to be slower than *in vivo* release. Similar results were obtained with lysozyme microspheres (75) (Fig. 12) and bovine-derived superoxide dismutase (bSOD) microspheres (124) (Fig. 13). In these studies, the authors believed that *in vivo* release was faster because of higher solubility of the drug in serum than in a buffer system, presence of hydrolytic enzymes *in vivo* along with formation of acidic microenvironment, and effect of plasma proteins on degradation of polyesters (75,117). However, slower *in vitro* release with orntide PLGA microspheres could be due to use of a small dialysis membrane surface area, which would increase equilibration time and would be responsible for lower peptide diffusion rates (116,117). In the study with lysozyme PLGA

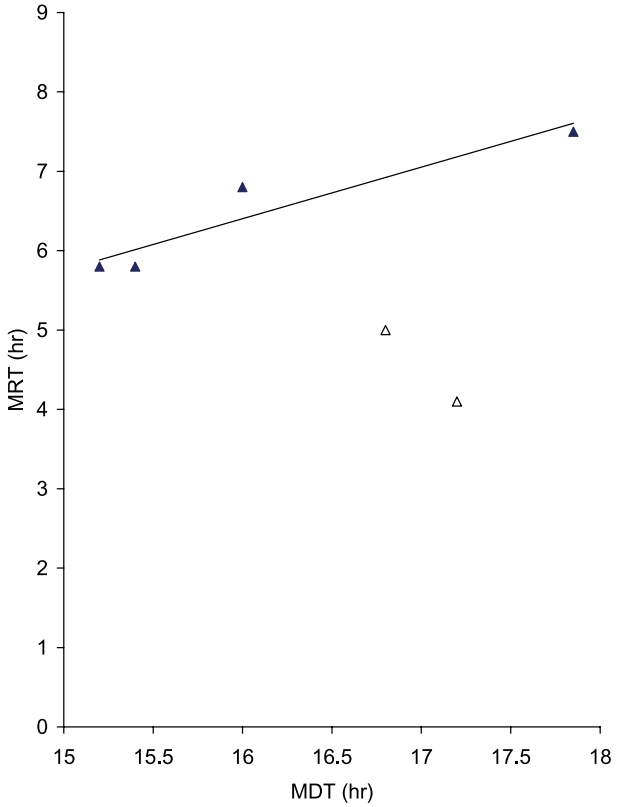

Fig. 14. Level B IVIVC for vapreotide PLGA microspheres [redrawn from Blanco-Prieto *et al.* (41)]. Filled triangles for end-group uncapped PLGA, and open triangles for end-group capped PLGA.

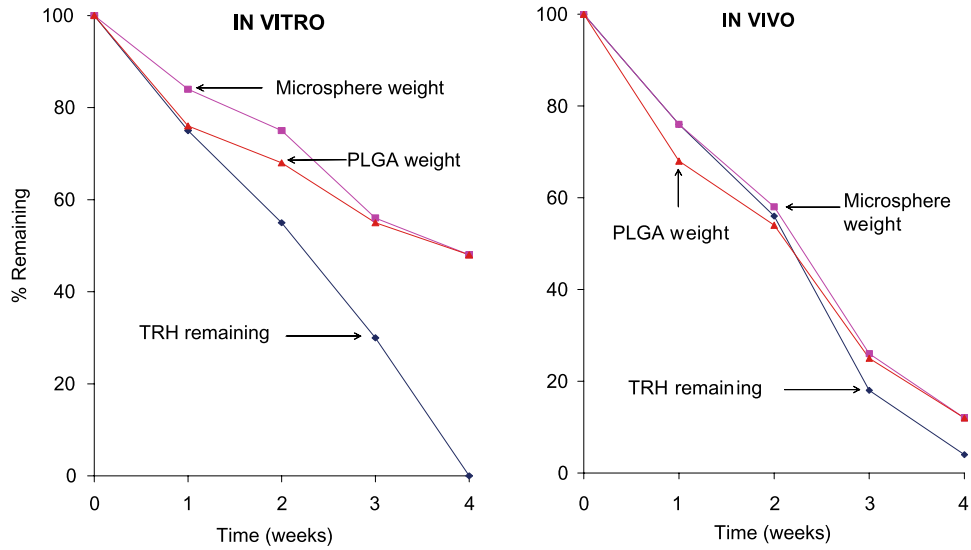

Fig. 15. Correlation of % remaining thyrotropin-releasing hormone (TRH), microsphere weight, and PLGA molecular weight for TRH PLGA microspheres [redrawn from Heya *et al.* (125)].

microspheres and bSOD microspheres (Figs. 12 and 13), slower *in vitro* release may be due to the low volumes used in the *in vitro* study (1.5 ml for lysozyme microspheres and 10 ml for bSOD microspheres). A higher concentration of polymer degradation products has been reported to lead to degradation of protein when small volumes of release media are used (37).

Two levels of correlation, B and C, of vapreotide microspheres (41) formulated using end-group capped (hydrophobic) and end-group uncapped (hydrophilic) PLGA polymers for 2- to 4-week delivery were studied. Level B IVIVC was based on statistical moment analysis, i.e., a comparison of mean dissolution time *in vitro* (MDT) with mean residence time *in vivo* (MRT), whereas level C was

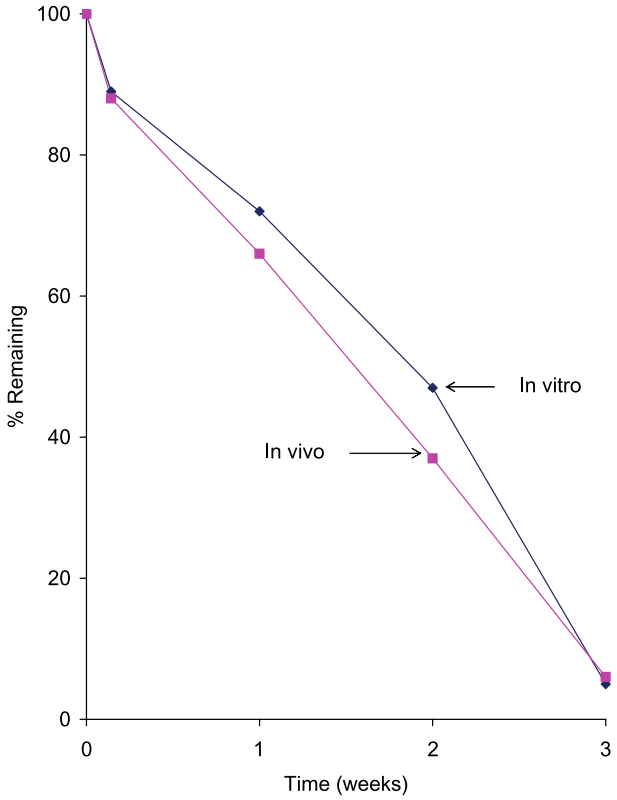

Fig. 16. Comparison of % drug remaining *in vivo* with *in vitro* for TRH PLGA microspheres [redrawn from Heya *et al.* (126)].

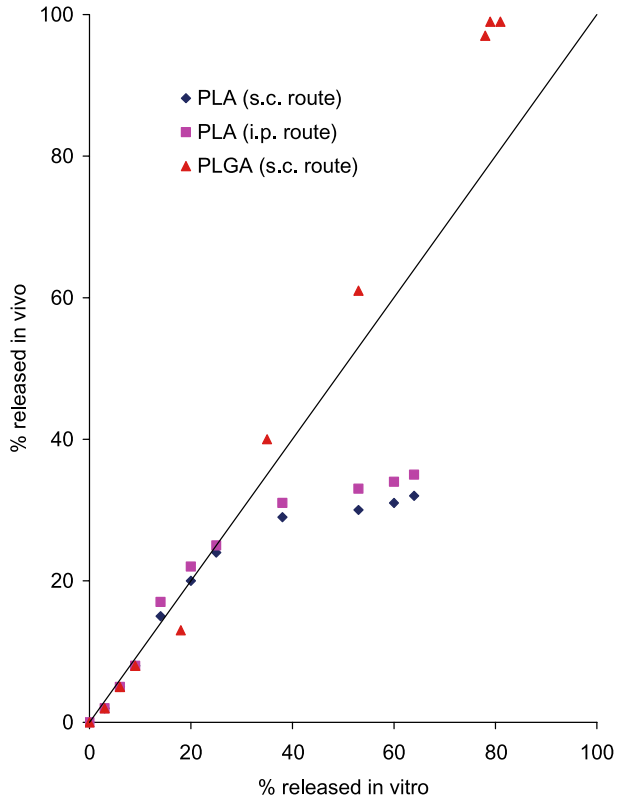

Fig. 17. IVIVC of methadone microspheres formulated using polylactide (PLA) and PLGA [from Negrin *et al.* (74)].

based on a single-point correlation between one dissolution time point and one pharmacokinetic parameter. Figure 14 shows the level B correlation attempted for the end-group uncapped and end-group capped PLGA polymers. Although the MRT lagged behind MDT values, a linear correlation (r = 0.958) between MDT and MRT was obtained for end-group uncapped PLGA. The authors reported a poor correlation for the end-group capped polymers (Fig. 14). A level C correlation (r = 0.932) for these formulations was obtained by comparing $AUC_{6\ h-14\ days}$ with the amount released *in vitro* for the same time period.

In a series of reports published by Heya *et al.* (125,126) on TRH formulated as microspheres using PLGA polymers (comonomer ratio 75:25) having MW 10 and 11 kDa, the rate and profile of % drug remaining *in vitro* were similar to those in rats *in vivo* (Figs. 15 and 16). Furthermore, the authors showed that mass loss (microsphere weight) and PLGA molecular weight decreased at a faster rate *in vivo* and was attributed to the faster absorption of acidic oligomers *in vivo*, which were probably not as soluble under *in vitro* conditions. Faster enzymatic degradation *in vivo* was also stated as a reason for higher mass loss. Comparable release rates *in vitro* and *in vivo* were attributed to the increased diffusion of the peptide *in vitro* because of greater physical destruction of the polymer caused by lower osmotic pressure of the release media and was confirmed with using scanning electron micrographs that showed an increase in porosity under *in vitro* conditions.

Negrin *et al.* (74) compared the IVIVC of a 7-day formulation of methadone microspheres formulated with D,L-PLA. Interestingly, serum levels of methadone obtained upon subcutaneous administration of the D,L-PLA microspheres in male Swiss mice were lower than expected from *in vitro* results with a bioavailability of 31.3%. Subsequent administration of the D,L-PLA microspheres by the intramuscular route revealed similar results (35.7% bioavailability). In an attempt to improve bioavailability, a formulation of methadone in PLGA was administered by the subcutaneous route. A bioavailability of 99.7% was obtained with the PLGA formulation. Attempts at IVIVC (Fig. 17) revealed a poor correlation for the PLA formulation, which was attributed to increased clearance of methadone, because of induction of its own metabolism *in vivo*.

CONCLUSIONS

This review illustrates that while the "sample and separate" method affords easy setup, sampling is cumbersome. Sampling with the "flow-through cell" method, although easier, requires a more time-consuming setup. Whereas the "dialysis" method has many variants, it seems to be a useful technique to study *in vitro* release from microparticulate delivery systems. Although the methods described in this review have not addressed nanoparticulate systems, the application to nanotechnology is obvious. In the opinion of the authors, the dialysis method would be quite applicable.

Although *in vitro* release tests can be used as an indicator of *in vivo* performance for microparticulate systems and also as a quality-control tool, the basis of selection of an *in vitro* release method is having an IVIVC. Future research with microparticulate delivery systems should focus on developing a release method that would be applicable for a wide spectrum of drug molecules and polymers.

REFERENCES

1. J. Schrier and P. DeLuca. Recombinant human bone morphogenetic protein-2 binding and incorporation in PLGA microsphere delivery systems. *Pharm. Dev. Technol.* **4**:611–621 (1999).
2. M. S. Hora, R. K. Rana, J. H. Nunberg, T. R. Tice, R. M. Gilley, and M. E. Hudson. Release of human serum albumin from poly(lactide-*co*-glycolide) microparticles. *Pharm. Res.* **7**:1190–1194 (1990).
3. Y. Capan, B. H. Woo, S. Gebrekidan, S. Ahmed, and P. P. DeLuca. Preparation and characterization of poly (D,L-lactide-*co*-glycolide) microspheres for controlled release of poly (L-lysine) complexed plasmid DNA. *Pharm. Res.* **16**:509–513 (1999).
4. J. L. Cleland, O. L. Johnson, S. Putney, and A. J. S. Jones. Recombinant human growth hormone poly(lactic-*co*-glycolic acid) microsphere formulation development. *Adv. Drug Deliv. Rev.* **28**:71–84 (1997).
5. H. Okada, M. Yamamoto, T. Heya, Y. Inoue, S. Kamei, Y. Ogawa, and H. Toguchi. Drug delivery using biodegradable microspheres. *J. Control. Release* **28**:121–129 (1994).
6. R. C. Mehta, R. Jeyanthi, S. Calls, B. C. Thanoo, K. W. Burton, and P. P. DeLuca. Biodegradable microspheres as depot system for parenteral delivery of peptide drugs. *J. Control. Release* **29**:375–384 (1994).
7. E. Esposito, R. Cortesi, and C. Nastruzzi. Gelatin microspheres: influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties. *Biomaterials* **17**:2009–2020 (1996).
8. K. Morimoto, H. Katsumata, T. Yabuta, K. Iwanaga, M. Kakemi, Y. Tabata, and Y. Ikada. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. *Eur. J. Pharm. Sci.* **13**:179–185 (2001).
9. K. Mladenovska, E. F. Kumbaradzi, G. M. Dodov, L. Makraduli, and K. Goracinova. Biodegradation and drug release studies of BSA loaded gelatin microspheres. *Int. J. Pharm.* **242**:247–249 (2002).
10. M. D. Blanco, M. V. Bernardo, C. Gomez, E. Muniz, and J. M. Teijon. Bupivacaine-loaded comatrix formed by albumin microspheres included in a poly(lactide-*co*-glycolide) film: *in vivo* biocompatibility and drug release studies. *Biomaterials* **20**:1919–1924 (1999).
11. K. Egbaria and M. Friedman. Release profiles of metronidazole and l-phenylalanine from individual albumin microspheres. *J. Control. Release* **14**:215–220 (1990).
12. D. J. Burgess and A. J. Hickey. *Microsphere Technology and Applications*, Marcel Dekker, New York, 1994.
13. T. Hickey, D. Kreutzer, D. J. Burgess, and F. Moussy. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. *Biomaterials* **23**:1649–1656 (2002).
14. J. C. Middleton and A. J. Tipton. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials* **21**:2335–2346 (2000).
15. L. Wu and J. Ding. *In vitro* degradation of three-dimensional porous poly(D,L-lactide-*co*-glycolide) scaffolds for tissue engineering. *Biomaterials* **25**:5821–5830 (2004).
16. M. Chaubal. Polylactides/glycolides—excipients for injectable drug delivery and beyond. *Drug Deliv. Technol.* **5**:34–36 (2002).
17. H. Okada. One- and three-month release injectable microspheres of the LH–RH superagonist leuprorelin acetate. *Adv. Drug Deliv. Rev.* **28**:43–70 (1997).
18. B. Ertl, P. Platzer, M. Wirth, and F. Gabor. Poly(D,L-lactic-*co*-glycolic acid) microspheres for sustained delivery and stabilization of camptothecin. *J. Control. Release* **61**:305–317 (1999).
19. J. M. Anderson and M. S. Shive. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Deliv. Rev.* **28**:5–24 (1997).

20. H. Okada and H. Toguchi. Biodegradable microspheres in drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **12**:1–99 (1995).
21. In L. Lachman, H. Lieberman, and J. Kanig (eds.), *The Theory and Practice of Industrial Pharmacy*, Lea & Febiger, PA, 1987.
22. D. J. Burgess, A. S. Hussain, T. S. Ingallinera, and M. L. Chen Assuring quality and performance of sustained and controlled release parenterals: workshop report. *AAPS PharmSci* **4**:E 7 (2002).
23. C. Washington. Drug release from microdisperse systems: a critical review. *Int. J. Pharm.* **58**:1–12 (1990).
24. U. S. P. 25. Rockville, MD, 2002, pp. 2011–2019.
25. C. Nastruzzi, E. Esposito, R. Cortesi, R. Gambari, and E. Menegatti. Kinetics of bromocriptine release from microspheres: comparative analysis between different *in vitro* models. *J. Microencapsul.* **11**:565–574 (1993).
26. B. Conti, I. Genta, P. Giunchedi, and T. Modena. Testing of "*in vitro*" dissolution behaviour of microparticulate drug delivery systems. *Drug Dev. Ind. Pharm.* **21**:1223–1233 (1995).
27. C. Gido, P. Langguth, J. Kreuter, G. Winter, H. Woog, and E. Mutschler. Conventional versus novel conditions for the *in vitro* dissolution testing of parenteral slow release formulations: application to doxepin parenteral dosage forms. *Pharmazie* **48**:764–769 (1993).
28. D. F. Bain, D. L. Munday, and A. Smith. Modulation of rifampicin release from spray-dried microspheres using combinations of poly-(D,L-lactide). *J. Microencapsul.* **16**:369–385 (1999).
29. D. J. Burgess, A. S. Hussain, T. S. Ingallinera, and M.-L. Chen. Assuring quality and performance of sustained and controlled release parenterals: AAPS Workshop Report, co-sponsored by FDA and USP. *Pharm. Res.* **19**:1761–1768 (2002).
30. D. J. Burgess, D. J. A. Crommelin, A. S. Hussain, and M.-L. Chen. Assuring quality and performance of sustained and controlled release parenterals. *Eur. J. Pharm. Sci.* **21**:679–690 (2004).
31. D. J. Burgess, A. S. Hussain, T. S. Ingallinera, and M. L. Chen. Assuring quality and performance of sustained and controlled release parenterals: Workshop Report. *AAPS PharmSci* **4**: article 7 (2002).
32. M. Siewert, J. Dressman, C. K. Brown, and V. P. Shah. FIP/AAPS guidelines to dissolution/*in vitro* release testing of novel/special dosage forms. *AAPS PharmSciTech* **4**: article 7 (2003).
33. M. Shameem, H. Lee, and P. P. DeLuca. A short term (accelerated release) approach to evaluate peptide release from PLGA depot-formulations. *AAPS PharmSci* **1**: article 7 (1999).
34. S. S. D'Souza, J. A. Faraj, and P. P. DeLuca. A model-dependent approach to correlate accelerated with real-time release from biodegradable. *AAPS PharmSciTech* **6**: article 70 (2005).
35. M. J. Schaefer and J. Singh. Effect of tricaprin on the physical characteristics and *in vitro* release of etoposide from PLGA microspheres. *Biomaterials* **23**:3465–3471 (2002).
36. G. Ruan and S.-S. Feng. Preparation and characterization of poly(lactic acid)–poly(ethylene glycol)–poly(lactic acid) (PLA–PEG–PLA) microspheres for controlled release of paclitaxel. *Biomaterials* **24**:5037–5044 (2003).
37. M. Diwan and T. G. Park. Stabilization of recombinant interferon-[alpha] by pegylation for encapsulation in PLGA microspheres. *Int. J. Pharm.* **252**:111–122 (2003).
38. F.-I. Liu, J. H. Kuo, K. C. Sung, and O. Y. P. Hu. Biodegradable polymeric microspheres for nalbuphine prodrug controlled delivery: *in vitro* characterization and *in vivo* pharmacokinetic studies. *Int. J. Pharm.* **257**:23–31 (2003).
39. G. Ruan, S.-S. Feng, and Q.-T. Li. Effects of material hydrophobicity on physical properties of polymeric microspheres formed by double emulsion process. *J. Control. Release* **84**:151–160 (2002).
40. N. Poulain, I. Dez, C. Perrio, M.-C. Lasne, M.-P. Prud'homme, and E. Nakache. Microspheres based on inulin for the controlled release of serine protease inhibitors: preparation, characterization and *in vitro* release. *J. Control. Release* **92**: 27–38 (2003).
41. M. J. Blanco-Prieto, M. A. Campanero, K. Besseghir, F. Heimgatner, and B. Gander. Importance of single or blended polymer types for controlled *in vitro* release and plasma levels of a somatostatin analogue entrapped in PLA/PLGA microspheres. *J. Control. Release* **96**:437–448 (2004).
42. M. Sivakumar and K. Panduranga Rao. Preparation, characterization and *in vitro* release of gentamicin from coralline hydroxyapatite–gelatin composite microspheres. *Biomaterials* **23**:3175–3181 (2002).
43. M. D. Dhanaraju, K. Vema, R. Jayakumar, and C. Vamsadhara. Preparation and characterization of injectable microspheres of contraceptive hormones. *Int. J. Pharm.* **268**:23–29 (2003).
44. S. B. Murty, J. Goodman, B. C. Thanoo, and P. P. DeLuca. Identification of chemically modified peptide from poly(D,L-lactide-*co*-glycolide) microspheres under *in vitro* release conditions. *AAPS PharmSciTech* **4**: article 50 (2003).
45. F.-L. Mi, S.-S. Shyu, Y.-M. Lin, Y.-B. Wu, C.-K. Peng, and Y.-H. Tsai. Chitin/PLGA blend microspheres as a biodegradable drug delivery system: a new delivery system for protein. *Biomaterials* **24**:5023–5036 (2003).
46. M. S. Latha, A. V. Lal, T. V. Kumary, R. Sreekumar, and A. Jayakrishnan. Progesterone release from glutaraldehyde cross-linked casein microspheres: *in vitro* studies and *in vivo* response in rabbits. *Contraception* **61**:329–334 (2000).
47. S.-Y. Yen, K. C. Sung, J.-J. Wang, and O. Yoa-Pu Hu. Controlled release of nalbuphine propionate from biodegradable microspheres: *in vitro* and *in vivo* studies. *Int. J. Pharm.* **220**:91–99 (2001).
48. Y.-I. Jeong, J.-G. Song, S.-S. Kang, H.-H. Ryu, Y.-H. Lee, C. Choi, B.-A. Shin, K.-K. Kim, K.-Y. Ahn, and S. Jung. Preparation of poly(-lactide-*co*-glycolide) microspheres encapsulating all-trans retinoic acid. *Int. J. Pharm.* **259**:79–91 (2003).
49. C. Volland, M. Wolff, and T. Kissel. The influence of terminal gamma-sterilization on captopril containing poly(-lactide-*co*-glycolide) microspheres. *J. Control. Release* **31**:293–305 (1994).
50. J. E. Rosas, R. M. Hernandez, A. R. Gascon, M. Igartua, F. Guzman, M. E. Patarroyo, and J. L. Pedraz. Biodegradable PLGA microspheres as a delivery system for malaria synthetic peptide SPf66. *Vaccine* **19**:4445–4451 (2001).
51. K. W. Burton, M. Shameem, B. C. Thanoo, and P. P. DeLuca. Extended release peptide delivery systems through the use of PLGA microsphere combinations. *J. Biomater. Sci., Polym. Ed.* **11**:715–729 (2000).
52. B. H. Woo, K.-H. Na, B. A. Dani, G. Jiang, B. C. Thanoo, and P. P. DeLuca. *In vitro* characterization and *in vivo* testosterone suppression of 6-month release poly(D,L-lactide) leuprolide microspheres. *Pharm. Res.* **19**:546–550 (2002).
53. S. Takada, T. Kurokawda, K. Miyazaki, S. Iwasa, and Y. Ogawa. Utilization of an amorphous form of a water-soluble GPIIb/IIIa antagonist for controlled release from biodegradable microspheres. *Pharm. Res.* **14**:1146–1150 (1997).
54. R. Bodmeier and J. W. McGinity. The preparation and evaluation of drug-containing poly(DL-lactide) microspheres formed by the solvent evaporation method. *Pharm. Res.* **4**: 465–471 (1987).
55. H. B. Ravivarapu, H. Lee, and P. P. DeLuca. Enhancing initial release of peptide from poly(DL-lactide-*co*-glycolide) (PLGA) microspheres by addition of a porosigen and increasing drug load. *Pharm. Dev. Technol.* **5**:287–296 (2000).
56. I. J. Castellanos, W. Al-Azzam, and K. Griebenow. Effect of the covalent modification with poly(ethylene glycol) on alpha-chymotrypsin stability upon encapsulation in poly(lactic-*co*-glycolic) microspheres. *J. Pharm. Sci.* **95**:327–340 (2005).
57. T. T. Thomas, D. S. Kohane, A. Wang, and R. Langer. Microparticulate formulations for the controlled release of interleukin-2. *J. Pharm. Sci.* **93**:1100–1109 (2004).
58. S. Özbas-Turan, J. Akbuga, and C. Aral. Controlled release of interleukin-2 from chitosan microspheres. *J. Pharm. Sci.* **91**:1245–1251 (2002).
59. P. K. Naraharisetti, M. D. Ning Lew, Y.-C. Fu, D.-J. Lee, and C.-H. Wang. Gentamicin-loaded discs and microspheres and their modifications: characterization and *in vitro* release. *J. Control. Release* **102**:345–359 (2005).
60. H. K. Kim and T. G. Park. Comparative study on sustained release of human growth hormone from semi-crystalline poly (lactic acid) and amorphous poly(,-lactic-*co*-glycolic acid)

microspheres: morphological effect on protein release. *J. Control. Release* **98**:115–125 (2004).
61. C. Berkland, M. J. Kipper, B. Narasimhan, K. K. Kim, and D. W. Pack. Microsphere size, precipitation kinetics and drug distribution control drug release from biodegradable polyanhydride microspheres. *J. Control. Release* **94**:129–141 (2004).
62. J.-C. Jeong, J. Lee, and K. Cho. Effects of crystalline microstructure on drug release behavior of poly([epsiv]-caprolactone) microspheres. *J. Control. Release* **92**:249–258 (2003).
63. L. Montanari, F. Cilurzo, F. Selmin, B. Conti, I. Genta, G. Poletti, F. Orsini, and L. Valvo. Poly(lactide-*co*-glycolide) microspheres containing bupivacaine: comparison between gamma and beta irradiation effects. *J. Control. Release* **90**:281–290 (2003).
64. C. Berkland, M. King, A. Cox, K. K. Kim, and D. W. Pack. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J. Control. Release* **82**:137–147 (2002).
65. Y.-Y. Yang, H.-H. Chia, and T.-S. Chung. Effect of preparation temperature on the characteristics and release profiles of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. *J. Control. Release* **69**:81–96 (2000).
66. C. Sturesson, P. Artursson, R. Ghaderi, K. Johansen, A. Mirazimi, I. Uhnoo, L. Svensson, A.-C. Albertsson, and J. Carlfors. Encapsulation of rotavirus into poly(lactide-*co*-glycolide) microspheres. *J. Control. Release* **59**:377–389 (1999).
67. F. W. Okumu, J. L. Cleland, and R. T. Borchardt. The effect of size, charge and cyclization of model peptides on their *in vitro* release from-PLGA microspheres. *J. Control. Release* **49**: 133–140 (1997).
68. R. T. Liggins and H. M. Burt. Paclitaxel loaded poly(L-lactic acid) microspheres: properties of microspheres made with low molecular weight polymers. *Int. J. Pharm.* **222**:19–33 (2001).
69. G. Wei, G. J. Pettway, L. K. McCauley, and P. X. Ma. The release profiles and bioactivity of parathyroid hormone from poly(lactic-*co*-glycolic acid) microspheres. *Biomaterials* **25**: 345–352 (2004).
70. T. G. Park, H. Yong Lee, and Y. Sung Nam. A new preparation method for protein loaded poly(D,L-lactic-*co*-glycolic acid) microspheres and protein release mechanism study. *J. Control. Release* **55**:181–191 (1998).
71. S.-A. Seo, G. Khang, J. M. Rhee, J. Kim, and H. B. Lee. Study on *in vitro* release patterns of fentanyl-loaded PLGA microspheres. *J. Microencapsul.* **20**:569–579 (2003).
72. H. Kim and D. J. Burgess. Effect of drug stability on the analysis of release data from controlled release of microspheres. *J. Microencapsul.* **19**:631–640 (2002).
73. H. Eroglu, H. S. Kas, L. Oner, O. F. Türkoglu, N. Akalan, M. F. Sargon, and N. Özer. The *in-vitro* and *in-vivo* characterization of PLGA: L-PLA microspheres containing dexamethasone sodium phosphate. *J. Microencapsul.* **18**:603–612 (2001).
74. C. M. Negrin, A. Delgado, M. Llabres, and C. Evora. *In vivo–in vitro* study of biodegradable methadone delivery systems. *Biomaterials* **22**:563–570 (2001).
75. G. Jiang, B. H. Woo, F. Kang, J. Singh, and P. P. DeLuca. Assessment of protein release kinetics, stability and protein polymer interaction of lysozyme encapsulated poly(D,L,-lactide-*co*-glycolide) microspheres. *J. Control. Release* **79**:137–145 (2002).
76. F.-X. Lacasse, P. HIldgen, J. Perodin, E. Escher, N. C. Phillips, and J. C. McMullen. Improved activity of an angiotensin receptor antagonist by spray-dried polymer microsphere preparation. *Pharm. Res.* **14**:887–891 (1997).
77. T. Heya, H. Okada, Y. Tanigawara, Y. Ogawa, and H. Toguchi. Effects of counteranion of TRH and loading amount on control of TRH release from copoly(DL-lactic/glycolic acid) microspheres prepared by an in-water drying method. *Int. J. Pharm.* **69**:69–75 (1991).
78. D. J. Burgess, S. S. Davis, and E. Tomlinson. Potential use of albumin microspheres as a drug delivery system. I. Preparation and *in vitro* release of steroids. *Int. J. Pharm.* **39**:129–136 (1987).
79. C. Washington and F. Koosha. Drug release from microparticulates; deconvolution of measurement errors. *Int. J. Pharm.* **59**:79–82 (1990).
80. A. Aubert-Pouëssel, D. C. Bibby, M.-C. Venier-Julienne, F. Hindre, and J.-P. Benoit. A novel *in vitro* delivery system for assessing the biological integrity of protein upon release from PLGA microspheres. *Pharm. Res.* **19**:1046–1051 (2002).
81. M. Kilicarslan and T. Baykara. The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. *Int. J. Pharm.* **252**:99–109 (2003).
82. A. Aubert-Pouëssel, M.-C. Venier-Julienne, A. Clavreul, M. Sergent, C. Jollivet, C. N. Montero-Menei, E. Garcion, D. C. Bibby, P. Menei, and J.-P. Benoit. *In vitro* study of GDNF release from biodegradable PLGA microspheres. *J. Control. Release* **95**:463–475 (2004).
83. R. Cortesi, E. Esposito, E. Menegatto, R. Gambari, and C. Nastruzzi. Gelatin microspheres as a new approach for the controlled delivery of synthetic oligonucleotides and PCR-generated DNA fragments. *Int. J. Pharm.* **105**:181–186 (1994).
84. R. Y. Cheung, R. Kuba, A. M. Rauth, and X. Y. Wu. A new approach to the *in vivo* and *in vitro* investigation of drug release from locoregionally delivered microspheres. *J. Control. Release* **100**:121–133 (2004).
85. N. Yuksel, E. Dinc, F. Onur, and T. Baykara. Influence of swelling degree on release of nicardipine hydrochloride from acrylic microspheres prepared by solvent evaporation method. *Pharm. Dev. Technol.* **3**:115–121 (1998).
86. C. Jollivet, A. Aubert-Pouëssel, A. Clavreul, M.-C. Venier-Julienne, S. Remy, C. N. Montero-Menei, J.-P. Benoit, and P. Menei. Striatal implantation of GDNF releasing biodegradable microspheres promotes recovery of motor function in a partial model of Parkinson's disease. *Biomaterials* **25**:933–942 (2004).
87. W. E. Longo and E. P. Goldberg. Hydrophilic albumin microspheres. *Methods Enzymol.* **112**:18–26 (1985).
88. B. W. Wagenaar and B. W. Muller. Piroxicam release from spray-dried biodegradable microspheres. *Biomaterials* **15**:49–54 (1994).
89. M. A. Vandelli, F. Rivasi, P. Guerra, F. Forni, and R. Arletti. Gelatin microspheres crosslinked with D,L-glyceraldehyde as a potential drug delivery system: preparation, characterisation, *in vitro* and *in vivo* studies. *Int. J. Pharm.* **215**:175–184 (2001).
90. B. H. Woo, J. W. Kostanski, S. Gebrekidan, B. A. Dani, B. C. Thanoo, and P. P. DeLuca. Preparation, characterization and *in vivo* evaluation of 120-day poly(D,L-lactide) leuprolide microspheres. *J. Control. Release* **75**:307–315 (2001).
91. K. Schultz, B. Mollgaard, S. Frokjaer, and C. Larsen. Rotating dialysis cell as *in vitro* release method for oily parenteral depot solutions. *Int. J. Pharm.* **157**:163–169 (1997).
92. D. H. Larsen, K. Fredholt, and C. Larsen. Assessment of rate of drug release from oil vehicle using a rotating dialysis cell. *Eur. J. Pharm. Sci.* **11**:223–229 (2000).
93. D. B. Larsen, S. Joergensen, N. V. Olsen, S. H. Hansen, and C. Larsen. *In vivo* release of bupivacaine from subcutaneously administered oily solution. Comparison with *in vitro* release. *J. Control. Release* **81**:145–154 (2002).
94. G. Lootvoet, E. Beyssac, G. K. Shiu, J.-M. Aiache, and W. A. Ritschel. Study on the release of indomethacin from suppositories: *in vitro–in vivo* correlation. *Int. J. Pharm.* **85**:113–120 (1992).
95. H. Parshad, K. Frydenvang, T. Liljefors, C. Cornett, and C. Larsen. Assessment of drug salt release from solutions, suspensions and *in situ* suspensions using a rotating dialysis cell. *Eur. J. Pharm. Sci.* **19**:263–272 (2003).
96. P. Saarinen-Savolainen, T. Jarvinen, H. Taipale, and A. Urtti. Method for evaluating drug release from liposomes in sink conditions. *Int. J. Pharm.* **159**:27–33 (1997).
97. S. Parsaee, M. N. Sarbolouki, and M. Parnianpour. *In-vitro* release of diclofenac diethylammonium from lipid-based formulations. *Int. J. Pharm.* **241**:185–190 (2002).
98. R. Bodmeier, H. Chen, P. Tyle, and P. Jarosz. Pseudoephedrine HCl microspheres formulated into an oral suspension dosage form. *J. Control. Release* **15**:65–77 (1991).
99. M. Y. Levy and S. Benita. Drug release from submicronized o/w emulsion: a new *in vitro* kinetic evaluation model. *Int. J. Pharm.* **66**:29–37 (1990).

100. T. M. Martin, N. Bandi, R. Shulz, C. B. Roberts, and U. B. Kompella. Preparation of budesonide and budesonide–PLA microparticles using supercritical fluid precipitation technology. *AAPS PharmSciTech* **3**: article 18 (2002).
101. E. Leo, R. Cameroni, and F. Forni. Dynamic dialysis for the drug release evaluation from doxorubicin–gelatin nanoparticle conjugates. *Int. J. Pharm.* **180**:23–30 (1999).
102. M. T. Peracchia, R. Gref, Y. Minamitake, A. Domb, N. Lotan, and R. Langer. PEG-coated nanospheres from amphiphilic diblock and multiblock copolymers: investigation of their drug encapsulation and release characteristics. *J. Control. Release* **46**:223–231 (1997).
103. J.-w. Nah, Y.-i. Jeong, and C.-s. Cho. Clonazepam release from core-shell type nanoparticles composed of poly(beta-benzyl L-glutamate) as the hydrophobic part and poly(ethylene oxide) as the hydrophilic part. *J. Polym. Sci., B, Polym. Phys.* **36**:415–423 (1998).
104. H.-J. Jeon, Y.-I. Jeong, M.-K. Jang, Y.-H. Park, and J.-W. Nah. Effect of solvent on the preparation of surfactant-free poly(lactide-*co*-glycolide) nanoparticles and norfloxacin release characteristics. *Int. J. Pharm.* **207**:99–108 (2000).
105. H. Heiati, R. Tawashi, R. R. Shivers, and N. C. Phillips. Solid lipid nanoparticles as drug carriers. I. Incorporation and retention of the lipophilic prodrug 3′-azido-3′-deoxythymidine palmitate. *Int. J. Pharm.* **146**:123–131 (1997).
106. A. K. Dash, P. W. Haney, and M. J. Garavalia. Development of an *in vitro* dissolution method using microdialysis sampling technique for implantable drug delivery systems. *J. Pharm. Sci.* **88**:1036–1040 (1999).
107. S. B. La, T. Okano, and K. Kataoka. Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)–poly(beta-benzyl L-aspartate) block copolymer micelles. *J. Pharm. Sci.* **85**:85–90 (1996).
108. J. Wang, B. M. Wang, and S. P. Schwendeman. Mechanistic evaluation of the glucose-induced reduction in initial burst release of octreotide acetate from poly(,-lactide-*co*-glycolide) microspheres. *Biomaterials* **25**:1919–1927 (2004).
109. W.-k. Lee, J.-y. Park, E. H. Yang, H. Suh, S. H. Kim, D. S. Chung, K. Choi, C. W. Yang, and J.-s. Park. Investigation of the factors influencing the release rates of cyclosporin A-loaded micro- and nanoparticles prepared by high-pressure homogenizer. *J. Control. Release* **84**:115–123 (2002).
110. S. Prabhu, J. L. Sullivan, and G. V. Betageri. Comparative assessment of *in vitro* release kinetics of calcitonin polypeptide from biodegradable microspheres. *Drug Deliv.* **9**:195–198 (2002).
111. J. Siepmann, N. Faisant, and J.-P. Benoit. A new mathematical model quantifying drug release from bioerodible microparticles using Monte Carlo simulations. *Pharm. Res.* **19**:1885–1893 (2002).
112. N. Faisant, J. Siepmann, and J. P. Benoit. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. *Eur. J. Pharm. Sci.* **15**:355–366 (2002).
113. N. Faisant, J. Siepmann, P. Oury, V. Laffineur, E. Bruna, J. Haffner, and J. P. Benoit. The effect of gamma-irradiation on drug release from bioerodible microparticles: a quantitative treatment. *Int. J. Pharm.* **242**:281–284 (2002).
114. C. Nastruzzi, C. Pastesini, R. Cortesi, E. Esposito, R. Gambari, and E. Menegatti. Production and *in vitro* evaluation of gelatin microspheres containing an antitumor tetra-amidine. *J. Microencapsul.* **11**:249–260 (1994).
115. J. Siepmann, N. Faisant, J. Akiki, J. Richard, and J. P. Benoit. Effect of the size of biodegradable microparticles on drug release: experiment and theory. *J. Control. Release* **96**:123–134 (2004).
116. J. W. Kostanski and P. P. DeLuca. A novel *in vitro* release technique for peptide containing biodegradable microspheres. AAPS PharmSciTech **1**: article 4 (2000).
117. J. W. Kostanski, B. A. Dani, G.-A. Reynolds, C. Y. Bowers, and P. P. DeLuca. Evaluation of orntide microspheres in a rat animal model and correlation to *in vitro* release profiles. *AAPS PharmSciTech* **1**: article 27 (2000).
118. J. Kostanski, B. C. Thanoo, and P. DeLuca. Preparation, characterization, and *in vitro* evaluation of 1- and 4-month controlled release orntide PLA and PLGA microspheres. *Pharm. Dev. Technol.* **5**:585–596 (2000).
119. T. G. Park, W. Lu, and G. Crotts. Importance of *in vitro* experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly(-lactic acid-*co*-glycolic acid) microspheres. *J. Control. Release* **33**: 211–222 (1995).
120. R. V. Diaz, M. Llabres, and C. Evora. One-month sustained release microspheres of 125I-bovine calcitonin: *in vitro–in vivo* studies. *J. Control. Release* **59**:55–62 (1999).
121. R. Kinget, A.-M. Bontinck, and H. Herbots. Problems of dialysis techniques in the study of macromolecule binding of drugs. *Int. J. Pharm.* **3**:65–72 (1979).
122. S. S. D'Souza and P. P. DeLuca. Development of a dialysis *in vitro* release method for biodegradable microspheres. *AAPS PharmSciTech* **6**: article 42 (2005).
123. V. R. S. Uppoor. Regulatory perspectives on *in vitro* (dissolution)/*in vivo* (bioavailability) correlations. *J. Control. Release* **72**:127–132 (2001).
124. T. Morita, Y. Sakamura, Y. Horikiri, T. Suzuki, and H. Yoshino. Evaluation of *in vivo* release characteristics of protein-loaded biodegradable microspheres in rats and severe combined immunodeficiency disease mice. *J. Control. Release* **73**:213–221 (2001).
125. T. Heya, Y. Mikura, A. Nagai, Y. Miura, T. Futo, Y. Tomida, H. Shimizu, and H. Toguchi. Controlled release of thyrotropin releasing hormone from microspheres: evaluation of release profiles and pharmacokinetics after subcutaneous administration. *J. Pharm. Sci.* **83**:798–801 (1994).
126. T. Heya, H. Okada, Y. Ogawa, and H. Toguchi. *In vitro* and *in vivo* evaluation of thyrotropin releasing hormone release from copoly(DL-lactic/glycolic acid) microspheres. *J. Pharm. Sci.* **83**: 636–640 (1994).