11
In Vitro–In Vivo Correlation on Parenteral Dosage Forms

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11.1 IVIVC Definition

In vitro and *in vivo* correlation (IVIVC) for drug products, especially for solid oral dosage forms, has been developed to predict product bioavailability from *in vitro* dissolution. Biological properties such as C_{max}, or AUC have been used to correlate with *in vitro* dissolution behavior such as percent drug release in order to establish IVIVC. IVIVC can be used to set product dissolution specifications; and as a surrogate for *in vivo* bioequivalence in the case of any changes with respect to formulation, process, or manufacturing site.

11.2 Modified Release Parenteral Products

Modified release (MR) parenteral products achieve sustained blood levels of therapeutics consequently decreasing dosing frequency and increasing patient compliance. These systems offer advantages over traditional dosage forms due to their sustained release capabilities and therefore more consistent blood levels that can result in a lowering of the systemic toxicity of drugs. The efficacy of chemotherapeutic agents has been reported to improve when steady relatively low blood levels were achieved compared to high dose i.v. bolus injections (Herben *et al.*, 1998; Hochster *et al.*, 1994). This can be accomplished by encapsulation of chemotherapeutics within liposomal and polymeric delivery systems. In addition, modified release parenteral products are used for targeted and localized drug delivery, which also reduces unwanted side effects.

Potential drug candidates for MR parenterals are chemotherapeutics or other drugs with a high incidence of adverse side effects; proteins or other macromolecules due to their instability in the gastrointestinal (GI) tract; drugs with short half-lives; drugs with low solubility; and drugs that are susceptible to high first-pass effect.

Modified release parenterals include: microspheres; liposomes; emulsions; suspensions; implants; drug eluting stents; and dendrimers. Recent developments in

synthetic chemistry have been utilized to make dendrimers, liposomes, and other parenteral delivery systems multifunctional through the addition of targeting moieties, and imaging agents. The reader is referred to the detailed reviews on the incorporation of monoclonal antibodies and other ligands to such delivery systems (Torchilin, 2005; Torchilin and Levchenko, 2003; Torchilin and Lukyanov, 2003). In addition, delivery system particle size and drug loading can be manipulated to alter tissue distribution as well as release rates. Surface modification with polyethylene glycol (PEG) or other polymers has been utilized to increase the blood circulation half-life.

11.3 Factors to Consider for Meaningful IVIVC

Strategies to develop meaningful IVIVC for MR products are summarized below. It is important first to obtain *in vivo* data, and then identify the *in vivo* drug release mechanism. The *in vitro* release method can then be designed with consideration to the *in vivo* release profile and mechanism.

11.3.1 Product Related Factors

There are several factors related to the formulation of MR parenterals that may affect the *in vivo* performance of these products when administered *via* parenteral routes (i.m., i.v., s.c., intra-CSF). These factors include formulation dispersibility, stability, injection volume, viscosity, and biocompatibility. To ensure dispersibility and also ease of injection, microspheres, and other dispersed system parenterals can be suspended in a vehicle containing an isotonic solution of carboxymethylcellulose, surfactant prior to administration (http://www.gene.com/gene/products/information/opportunistic/nutropin-depot/insert.jsp). The injection of a homogenous suspension of microspheres should be assured otherwise erroneous dosing may occur that would affect the *in vivo* data and hence the development of an IVIVC. On the other hand, the presence of surfactant could affect the release properties *in vivo* by enhancing drug solubility and diffusion or affecting viscosity. It has been reported that variation in the injection depth for i.m. administration resulted in large variations in plasma drug concentrations (Zuidema *et al.*, 1994). Formulation stability should be monitored prior to injection of dispersed systems since any particle size change may result in adverse effects and alteration of drug release characteristics. Another important factor with respect to microspheres is the reconstitution time since premature drug release may occur in the delivery vehicle due to dissolution of surface associated drug from the microspheres. This may result in an underestimation of the initial dose released (burst release) upon administration.

Nonionic surfactants such as Cremophor®EL (CrEL; polyoxyethyleneglycerol triricinolate 35) and polysorbate 80 (Tween 80) have been used to solubilize a variety of drugs prior to i.v. administration. A detailed review by Tije *et al.* reports

adverse effects such as acute hypersensitivity and peripheral neurotoxicity as well as altered pharmacokinetics of chemotherapeutics when administered with these surfactants (ten Tije *et al.*, 2003). In addition, there may be toxicity issues with certain excipients, especially when used at high concentration. For example, administration of propylene glycol at concentrations above 40% has been reported to cause muscle damage. Consequently, *in vivo* markers, such as cytosolic enzymes, creatine kinase and lactate dehydrogenase, should be monitored as these are indicators of tissue damage which may result from either the drug, or the excipients. The encapsulated drug formulation may result in a reduction in toxicity, for example microspheres or liposomes can be used to isolate high concentrations of irritant drugs which are then released slowly at levels that either do not show toxicity or show limited toxicity *in vivo*. For example, it has been shown that encapsulation of tissue irritant drugs into liposome formulations reduced muscle damage considerably (Kadir *et al.*, 1999). Toxicity and irritancy at the *in vivo* site can affect drug release due to resulting edema as well as the presence of increased numbers of neutrophils and macrophages.

The different manufacturing techniques used to prepare polymeric delivery systems as well as liposomes mostly involve the use of organic solvents. The processes of removal of organic solvent and of determining the amount of residual solvent in the product are crucial due to the *in vivo* relevance (toxicity, tolerance, systemic side effects).

11.3.2 Factors Affecting In Vitro Release

In vitro release methods are an integral part of the product development process to establish quality, performance, and batch to batch consistency as well as *in vivo* and *in vitro* relationships. Current uses of *in vitro* release testing are summarized in Table 11.1.

Unfortunately, there is a lack of standards or guidance documents for *in vitro* release testing methods for modified release parenterals. The United States Pharmacopeia (USP) apparatus for dissolution testing methods were developed for solid oral dosage forms and transdermal products. Briefly, USP Apparatus 1 (basket) and 2 (paddle) are suitable for solid dosage forms. Apparatus 3 (reciprocating cylinder) and Apparatus 4 (flow-through cell) were developed for drugs with limited solubility and are useful for MR products. Apparatus 5 (paddle over disc), Apparatus 6 (cylinder), and 7 (reciprocating disk) were developed for transdermal delivery systems. In some cases current USP methods have been modified to overcome limitations of the existing methods for application to MR parenterals

TABLE 11.1. Current uses of *in vitro* release testing method

- Formulation development
- Quality assurance and process control
- Evaluation of the changes in the manufacturing process
- Substantiation of label claims
- Compendial testing

products. For example, USP Apparatus 4 has been adapted for microsphere testing through the inclusion of glass beads in the flow-through cells (Zolnik *et al.*, 2005). The glass beads are interspersed between the microspheres to prevent aggregation during the release study and to more closely simulate the *in vivo* conditions where the microspheres are interspersed among the cells, e.g, at the s.c. site (Zolnik *et al.*, 2005). Moreover, the addition of the glass beads in the flow-through cells allows laminar flow of release media and prevents the formation of channels in the solid bed where the media flow through while other areas in the bed would remain unwetted. Figure 11.1 displays the schematic diagram of flow-through cell containing microspheres and glass beads in the closed mode.

In vitro release testing methods currently used in research and development as well as quality control include: dialysis sac, sample-and-separate, ultrafiltration, continuous flow methods, and microdialysis. The dialysis sac method involves suspending microspheres or other dispersed systems in a dialysis sac with a semipermeable membrane that allows diffusion of the drug, and then drug concentration is monitored in the receiver chamber. Disadvantages of this method include (a) potential for dispersed system aggregation due to the lack of agitation and (b) violation of sink conditions may result when drug release from the microspheres is faster than drug diffusion through the membrane (Chidambaram and Burgess, 1999). A reversed dialysis method has been developed by Chidambaram and Burgess, where the dispersed phase is placed in the large chamber with the media and the sacs contain only media. The sacs are then sampled at the different time points. This method overcomes the problem of violation of sink conditions (Chidambaram and Burgess, 1999). The sample and separate technique utilizes USP Apparatus 2 (paddle method) where microspheres are dispersed in the media

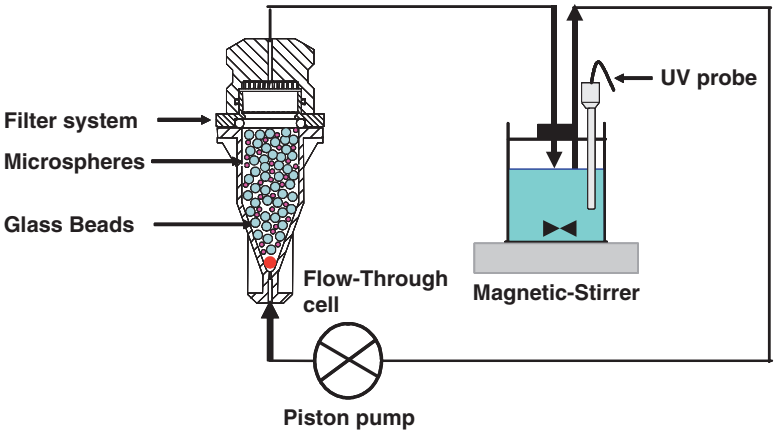

FIGURE 11.1. Schematic diagram of 12 mm flow-through cell containing microspheres and glass beads in the flow-through method (closed system). Placement of the fiber optic probe in the reservoir vessel is also shown

and at different time points samples are withdrawn, separated *via* ultracentrifugation or filtration and the filtrate is analyzed for drug content with an appropriate analytical method. The disadvantages of this method are the difficulty in separation of the delivery system from the media, for example, ultrafiltration requires 1 or 2 h at high centrifugational force ($150,000 \times g$) and this often is an undesirable method due to disruption of the delivery system and consequent alteration in the release pattern (Chidambaram and Burgess, 1999). As an alternative, low pressure ultrafiltration has been used to prevent disruption. The disadvantage of this method is the lack of available membranes with appropriate cut off points since some delivery systems are in the submicron and micron size range (Magenheim *et al.*, 1993). The continuous flow method (USP Apparatus 4) consists of a reservoir, a pump and flow-through cells where the microspheres or other dispersed systems are contained. The continuous flow method avoids problems associated with separation of the dispersed system from the media since the dispersed system is isolated in the flow-through cells and the media can be sampled from the reservoir. Another advantage of the flow-through method for dispersed systems is that since the dispersed system is isolated from the media reservoir this allows *in situ* monitoring. UV fiber optic probes can be placed in the media reservoir vessel thus avoiding the potential problem of interference from dispersed system particles sticking to the probe. *In situ* monitoring has the advantage that multiple time points can be analyzed to allow for complete characterization of the release profile. For example, this method has been used to characterize the burst release phase from microspheres. Schematic showing the placement of the *in situ* probes is shown in Fig. 11.1 (Zolnik *et al.*, 2005). In addition, violation of sink conditions for drugs with limited solubility is not an issue with the continuous flow USP 4 method due to the ease of media replacement.

Microdialysis has been used to study pharmacokinetics of drugs in peripheral tissues (Boschi and Scherrmann, 2000; de la Pena *et al.*, 2000). Recently microdialysis has been used to monitor drug release *in vitro* (Dash *et al.*, 1999). The basic principle of this technique is to measure drug release continuously from an implant site by mimicking a capillary blood vessel with a thin dialysis tube. An advantage of this technique is that the flow rate of the media can be adjusted to as low as 0.5 μl/min. Other advantages of this technique are (a) small volume (b) continuous monitoring of drug release, and (c) online analysis (Dash *et al.*, 1999). Dash *et al.* had compared a microdialysis method with the USP Apparatus 3 method to monitor ciprofloxacin release from PLGA implants and reported that both these methods were in close agreement. Researchers have also evaluated miniaturized methods where small volumes of media are employed due to the *in vivo* relevance (volume at the s.c. site is low). However, the disadvantages of this method are violation of sink conditions and the potential for dispersed system aggregation due to the limited volume and lack of agitation.

In order to develop meaningful IVIVC, study design for *in vitro* release should be performed after *in vivo* data are available, so that media conditions can be manipulated to mimic the *in vivo* behavior. To this end, researchers

have investigated different media conditions to aid in the development of a relationship between *in vivo* and *in vitro* release data. The use of cosolvent, addition of surfactants and enzymes, variation in pH, ionic strength, agitation and temperature have been investigated (Agrawal *et al.*, 1997; Aso *et al.*, 1994; Blanco-Prieto *et al.*, 1999; Hakkarainen *et al.*, 1996; Jiang *et al.*, 2002; Li *et al.*, 2000; Makino *et al.*, 1986). For example, acidic media have been used to mimic drug release from PLGA microspheres *in vivo* (Blanco-Prieto *et al.*, 1999; Heya *et al.*, 1994a).

There is no single *in vitro* release testing method suitable for all parenterals delivery systems due to their complexities. However, USP Apparatus 4 is recommended for modified release oral formulations and is appropriate for modified release parenterals. USP Apparatus 4 has been recommended for MR microsphere products (Burgess *et al.*, 2004). The physicochemical properties of drugs and delivery systems should be taken into account when choosing an appropriate release method. In addition, the *in vitro* method should be able to discriminate between formulations with different *in vivo* release characteristics.

11.3.2.1 Accelerated *In Vitro* Release Testing

Since MR parenterals may be intended to release drug for days, weeks, and even months, accelerated *in vitro* release testing methods are required for routine testing of these products. Therefore, if the accelerated method is to be used as a surrogate for *in vivo* studies IVIVC must be established using the accelerated method. A problem here is that accelerated methods, by their nature, often change the mechanism of drug release and this can make the establishment of an IVIVC more difficult. For example, elevated temperature accelerated conditions have been shown to alter the mechanism of release from PLGA microspheres from degradation controlled to diffusion controlled (Zolnik *et al.*, 2006). On the other hand, under pH accelerated conditions, release from PLGA microspheres appeared to be degradation controlled eventhough morphological changes occurred during degradation that were distinctly different from those that occur during "real-time" *in vitro* release testing.

11.3.3 Mathematical Models of In Vitro Drug Release

Different models have been developed depending on the governing, rate-limiting step of drug release. For MR systems the mathematical models used can be categorized as: diffusion controlled, swelling controlled, and erosion controlled release systems.

Mathematical models to evaluate drug release have been extensively used, especially for solid dosage forms to understand drug transport through barriers. Fick's second law of diffusion states that the rate of change in concentration is proportional to the rate of change in the concentration gradient at that point where the proportionality constant is equal to the diffusivity "D". The assumption is

constant diffusivity.

$$\frac{\mathrm{d}C}{\mathrm{d}t} = D\left[\frac{\mathrm{d}^2C}{\mathrm{d}x^2} + \frac{\mathrm{d}^2C}{\mathrm{d}y^2} + \frac{\mathrm{d}^2C}{\mathrm{d}z^2}\right] \tag{11.1}$$

Various exact solutions of (11.1) depending on the boundary condition of the system were reviewed in detail by Flynn *et al.* (1974). The commonly used form of (11.1) is below. The assumptions necessary to arrive at (11.2) are (a) sink conditions are maintained; (b) diffusivity is constant; and (c) steady state is reached.

$$\frac{\mathrm{d}M}{\mathrm{d}t} = \frac{DC_0}{h} \tag{11.2}$$

Higuchi derived the following equation for systems when boundaries change with time, such as drug release from a semisolid ointment. The change in the amount released per unit area, $\mathrm{d}M$, is equal to a change in the thickness of the moved boundary, $\mathrm{d}h$. A is the total amount of drug in the matrix. C_s is the saturation concentration of the drug within the matrix

$$\mathrm{d}M = A\,\mathrm{d}h - \frac{C_\mathrm{s}}{2}\mathrm{d}h. \tag{11.3}$$

According to Ficks law, $\mathrm{d}M$ is equal to (11.2). The equation which describes the amount released as a linear function of the square root of time can be derived (11.4) after setting (11.2) and (11.3) equal. The assumptions used in this derivation are: initial drug loading is much higher than drug solubility, swelling of the system is negligible, sink conditions are maintained, and edge effects are negligible.

$$M = \sqrt{2C_\mathrm{s}DA}. \tag{11.4}$$

There are several mathematical models derived for different systems and different geometries (such as, spheres), as well as for release of drugs suspended in spherical particles, and for systems where the rate of drug release is swelling controlled.

Ritger and Peppas (1987) derived a semiempirical equation known as the power law (11.5) for systems with different geometries (slab, cylinder, and sphere) to describe drug release for diffusion controlled, swelling controlled, and controlled by intermediate anomalous mass transport.

$$\frac{M_t}{M\infty} = kt^n, \tag{11.5}$$

where k is a constant and n is the release exponent indicative of the drug release mechanism. In the case of Fickian diffusion controlled release, n equals to 0.43 for spherical geometry.

In order to identify the drug release mechanism from low molecular weight PLGA microspheres, (11.3) was utilized. Diffusion kinetics were confirmed for different flow rates using modified USP Apparatus 4 (Zolnik *et al.*, 2006). Modeling of drug release from biodegradable polymers such as PLGA is complex since

it involves not only diffusion phenomena of drug release but also physicochemical changes in the polymer. Empirical models have been derived based on the assumption that one net mechanism with zero order process can describe all mechanisms involved, such as dissolution, swelling, and polymer degradation. Mechanistic models based on Monte Carlo simulations have been applied to describe polymer degradation and diffusion phenomena (Siepmann and Gopferich, 2001). Drug release from such systems has also been modeled by including the dependence of the diffusion coefficient on the polymer molecular weight change (Faisant *et al.*, 2002). Lemaire *et al.* were able to show the relative dominance between the diffusion and erosion release kinetics when different parameters such as erosion rate, initial pore size, porosity and the diffusion coefficient of the drug were varied (Lemaire *et al.*, 2003).

It has been established that PLGA degradation followed pseudo-first-order degradation kinetics (11.6).

$$Mw(t) = Mw_0 e^{-k_{deg}t} \tag{11.6}$$

First order degradation kinetics have been observed from PLGA microspheres at elevated temperature. This was used to establish drug release mechanisms under accelerated release conditions where temperature varied between 37 and 70°C (Zolnik *et al.*, 2006). It should be noted that when an initial burst release exists, it is recommended to test the burst phase separately under "real-time" conditions, as under accelerated conditions the burst phase is usually not observed. Likewise it is often necessary to model the release separately from the burst phase. High correlation has been observed for drug release from PLGA microspheres postburst release (Zolnik *et al.*, 2006).

11.3.4 Factors Affecting In Vivo Release

In vivo release from MR parenterals such as microspheres may be affected by the environment at the site of administration for example s.c. or i.m. injected products are generally retained at the administration site depending on the particle size. *In vivo* factors that affect drug release can be classified as delivery system independent and delivery system dependent. Delivery system independent factors include barriers to drug diffusion (e.g., fluid viscosity and connective tissue); drug partitioning at the site (e.g., uptake into fatty tissue); available fluid volume at the site; and in the case of intramuscular injection muscle movement may also be an important factor. For example, factors related to subcutaneous tissue are interstitial fluid volume, blood flow rate, osmotic pressure, and the presence of plasma proteins. It has been reported that the diffusion of macromolecules from the interstitium may be delayed by the fibrous collagen network, and the gel structure of proteoglycans as well as possible electrostatic interaction with components of the interstitium. More information on protein absorption and bioavailability from the subcutaneous tissue can be found in a detailed review article by Porter and Charman (2000). Delivery system dependent factors are those specific to a particular delivery system and include enzymatic degradation of susceptible polymers, protein adsorption, phagocytosis

as well as inflammatory reaction. For example, the initial acute phase of inflammation results in an influx of fluid together with phagocytic cells and the increased fluid volume may increase drug release and adsorption. Whereas, the chronic stage of inflammation can lead to fibrosis which in turn results in isolation of the delivery system with consequent reduction in the fluid volume. A major challenge to *in vivo* delivery of drug carriers following IV administration is the rapid removal of these particles from circulation by the reticuloendothelial system (RES) mainly the Kupffer cells of the liver and the macrophages of the spleen and bone marrow. In order to reduce interaction with plasma proteins and consequently prevent RES uptake, and increase blood circulation time, MR parenterals have been surface modified with PEG polymers. A thorough review on this subject can be found in an article by Moghimi *et al.* (2001).

Different drugs have been coencapsulated in microspheres to alter their *in vivo* behavior. For example, dexamethasone was coencapsulated with bupivacaine to increase the concentration of bupivacaine at the local site by decreasing its clearance from the tissues due to the vasoconstrictive nature of dexamethasone. In this case, *in vitro* release of bupivacaine was not altered when dexamethasone was incorporated. Care should be taken to determine the pharmacodynamic effects when drugs are given in combination in such formulations (McDonald *et al.*, 2002).

11.4 *In Vitro–In Vivo* Correlation

IVIVC can be categorized as follows: Level A, point-to-point correlation over the entire release profile and is used to claim biowaivers; Level B, mean *in vitro* dissolution time is compared to either the mean residence time or the mean *in vivo* dissolution time; Level C, single point correlation between a dissolution parameter (for example, the amount dissolved at a particular time or the time required for *in vitro* dissolution of a fixed percentage of the dose) and an *in vivo* parameter (for example, C_{max} or AUC); Multiple Level C correlation, a Level C correlation at several time points in the release profile.

Figure 11.2 summarizes general considerations with respect to *in vivo* release and distribution of protein loaded microspheres for establishing IVIVC. In this scheme Morita *et al.* compartmentalized the events involving *in vivo* pharmacokinetics of protein release from microspheres as: drug release rate constants (K_{rel}) from microspheres, protein degradation constant as K_{deg}, drug absorption to systemic circulation defined as K_a, and distribution to target tissues as K_d while drug elimination from kidney or liver defined as K_{el}. In this scheme, Morita *et al.* have also included the possible immune response effects on *in vivo* pharmacokinetics of proteins due to generation of specific antibodies. The authors indicated that antibodies generated in normal mice may alter the clearance rate of bovine derived superoxide dismutase and this affect was not observed in severe combined immunodeficiency disease mice. In this scheme, there are three output functions which are used to establish IVIVC, X1 *in vitro* release profile correlated to either Y1

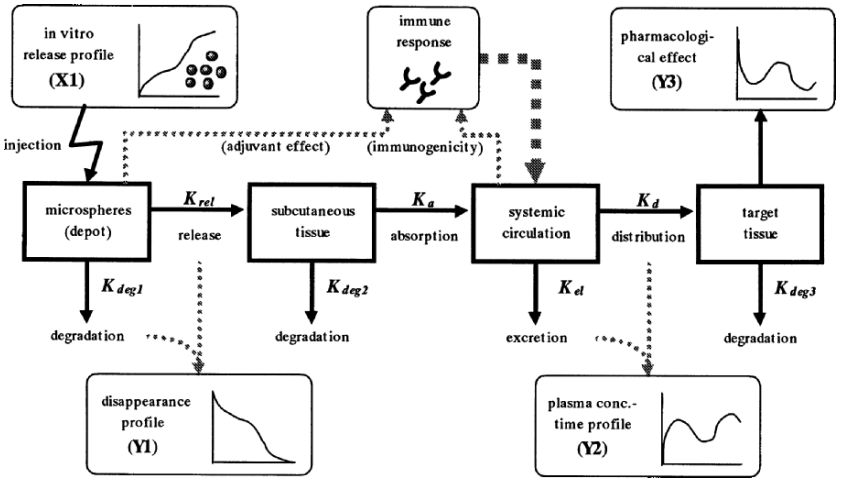

FIGURE 11.2. General considerations for the IVIVC of protein loaded microspheres

defined as disappearance profile from the administration site, or plasma concentration time profile as Y2. The pharmacological effects of drugs at the target tissue are defined as Y3 (Morita *et al.*, 2001). Different levels of correlations can be achieved by comparing X1 (*in vitro* drug release) to Y1 (*in vivo* disappearance) or Y2 (plasma concentration time profile). If Y2 is used, convolution procedure or any other modeling technique can be used to relate plasma concentration time profile to *in vivo* absorption or release rate. If a linear relationship between the *in vitro* and release data does not occur then, IVIVC can be achieved by mathematical modeling (e.g. time variant nonlinear modeling) of the *in vitro* and *in vivo* data (Young *et al.*, 2005).

11.5 Microspheres

Microspheres are polymeric spherical particles in the micron size range. Drug can be entrapped in these particles either in the form of microcapsules with a polymer coating surrounding a drug core or in the form of micromatrices with the drug dispersed throughout the polymer (Burgess and Hickey, 1994). Both natural and synthetic polymers have been used to form microspheres (Cleland, 1997). In this chapter, synthetic polymers such polyesters, poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) polymers (PLGA), will be reviewed. These polymers gained importance in the field of drug delivery due to their biodegradability and relative biocompatibility (Kulkarni *et al.*, 1971). Lupron Depot®, that releases potent analogue of luteinizing hormone–releasing hormone (LH–RH) over periods of 1 and 3 months (Okada, 1997), was the first controlled release microsphere product available on the US market for the treatment of hormone dependent

prostate and mammary tumors, and endometriosis. Since 1989, the Food and Drug Administration has approved the following five PLGA microsphere products (Lupron Depot, Sandostatin LAR, Nutropin Depot and Trelstar Depot, Risperdal Consta). Microspheres are designed as modified release drug delivery systems where drug is released in periods of days to months. From a safety and efficacy perspective, it is important to understand drug release kinetics from such formulations. Microsphere systems tend to exhibit complex release kinetics with: an initial burst release, as a result of surface associated drug and this is usually diffusion controlled. Following the burst release phase, the mechanism of release may be diffusion or erosion controlled or a combination of thereof (Gopferich, 1996; Lewis, 1990; Okada, 1997). Drug release from PLGA microspheres typically falls under the combination of diffusion and erosion controlled where an initial burst release is followed by a lag phase and then a secondary, apparent zero order release phase. The lag phase is considered to be a result of the time required for the build up of acid byproducts, and, hence for sufficient bulk erosion to take place, to increase porosity and allow for the subsequent secondary apparent zero order phase (Brunner *et al.*, 1999; Mader *et al.*, 1998; Shenderova *et al.*, 1999).

In the literature different levels of IVIVC have been established for PLGA microspheres. In a study by Zolnik and Burgess, a biorelevant *in vitro* release method (USP 4 method) and Sprague Dawley rat model was utilized to obtain a relationship between *in vitro* and *in vivo* release of dexamethasone from two different PLGA microsphere formulations. A linear IVIVC using the time shifting/scaling method discussed above was established for microsphere formulations prepared with different molecular weights of PLGA. The time scaling/shifting method was applied to *in vitro* data due the observance of faster *in vivo* release of dexamethasone (Zolnik 2005). In addition, the release of dexamethasone was able to control the inflammatory reaction that would otherwise occur to the presence of microspheres and to the tissue damage that occurs due to needle injection and this appeared to result in faster *in vivo* kinetics compared to the *in vitro* kinetics. In a previous publication from our laboratory, it has been reported that release kinetics of vascular endothelial growth factor (VEGF) from PLGA microspheres was slower *in vivo* compared to *in vitro* (Kim and Burgess, 2002). A possible explanation for this is the severe inflammatory reaction that occurred in the presence of these microspheres. This is considered to be a result of both tissue reaction to the PLGA microspheres as well as to the foreign protein (human VEGF was used in a rat model).

It has also been shown for leuprolide that repeated injections of Lupron depot did not alter the bioavailability and urinary excretion of leuprolide and the mean serum levels and AUC of Lupron Depot, correlated linearly with each dose. More detailed information on the formulation, drug release and *in vivo* animal models of leuprorelin depot can be found in a comprehensive review by Okada (1997). The evaluation of different *in vitro* conditions to mimic *in vivo* release of thyrotropin releasing hormone (TRH) from PLGA microspheres were investigated by Heya *et al.* (1994a). Authors concluded that the selection of the media conditions (such as medium pH, buffer concentration, ionic strength) is important to obtain an

in vitro release profile that mimics *in vivo* release, especially for hydrophilic drugs (Heya *et al.*, 1994a). In the follow-up study, Heya *et al.* examined the pharmacokinetics of TRH from PLGA microspheres and determined that sustained *in vitro* release kinetics were mimicked *in vivo* using 33 mM pH 7 phosphate buffer containing 0.02% Tween 80 (Heya *et al.*, 1994b).

Other examples of Level A correlation were demonstrated by Cheung *et al.* and utilized the continuous flow method in dynamic and static mode to mimic *in vivo* release from locoregionally administered dextran-based microspheres (Cheung *et al.*, 2004). An example of Level B correlation was shown for release of the somatostatin analogue vapreotide from PLA and PLGA microspheres where the mean *in vivo* residence time was correlated with the mean *in vitro* dissolution time (Blanco-Prieto *et al.*, 2004).

A linear IVIVC was demonstrated in a different polymer system by van Dijkhuizen-Radersma (2004) in a study of protein release from poly(ethylene glycol) terephthalate (PEGT)/poly(butylene terephthalate) PBT microspheres. Similar to the properties of polyesters, poly(ether–ester) PEGT/PBT multiblock copolymers exhibit biodegradability and biocompatibility. Three different microsphere formulations with varied PEGT/PBT weight ratio and PEG segment length were investigated. The diffusion coefficient of drugs from PEGT/PBT microspheres was dependent on polymer swelling which in turn was related to its PEG segment length. *In vitro* release from PEGT/PBT microspheres correlated with the volume swelling ratios, faster release was obtained using polymers with higher swelling ratios.

In vivo release kinetics are often not predicted by *in vitro* release methods, possibly due to selection of inappropriate *in vitro* release conditions, and methods (Diaz *et al.*, 1999; Jiang *et al.*, 2003). It should be also noted that inappropriate selection of animal model may result in unsuccessful IVIVC. For example, Perugini *et al.* demonstrated that a rat model was not suitable to induce osteopenia and therefore, IVIVC could not be established (Perugini *et al.*, 2003). The difficulties in determination of drug amounts in the biological matrix also resulted in lack of IVIVC (Yenice *et al.*, 2003). In addition, there are several comprehensive and well-designed research articles in the literature on the evaluation of *in vitro* and *in vivo* release of drugs from microspheres; however, these articles did not attempt to show any mathematical correlation of their *in vivo* and *in vitro* results (Liu *et al.*, 2003).

11.6 Liposomes

Liposomes consist of one or more phospholipid bilayers with enclosed aqueous phase. Depending on the method of preparation of liposomes, different types of liposomes are formed: large multilamellar (MLVs); small unilamellar, (SUVs); or large unilamellar (LUVs). Liposomal drug delivery has advantages over traditional therapy in cancer treatment due to increased tumor uptake *via* enhanced permeation and retention (EPR) effect where tumor tissue has leaky vasculature

TABLE 11.2. MR products in the market

Active drug	Product name	Indications
Microsphere products		
Leuprolide	Lupron	Endometriosis
Octreotide	Sandostatin LAR	Agromegaly
Somatropin	Nutropin depot	Growth therapy
Triptorelin	Triptorelin	Prostate cancer
Abarelix	Plenaxis	Prostate cancer
Liposome products		
Daunorubicin	DaunoXome	Kaposi's sarcoma
Doxurubicin	Mycet	Combinational therapy of recurrent breast cancer
Doxurubicin in PEG-liposomes	Doxil/Caelyx	Refractory Kaposi's sarcoma; ovarian cancer; recurrent breast cancer
Amphotericin B	AmBiosome	Fungal infection
Cytarabine	DepoCyt	Lymphomatous meningitis
Vincristine	Onco TCS	Non-Hodgkin's lymphoma
Emulsion products		
Propofol	Diprivan	Anesthetic
Diazepam	Dizac	Epilepsy

and poor lymphatic drainage (Maeda *et al.*, 2001). Liposomal products with encapsulated daunorubicin, doxorubicin, and vincristine are currently in the market for the treatment of cancer (Table 11.2). Drug release and cell uptake kinetics may depend on the size, charge, surface properties of the liposomes as well as on the types of lipids used. The incorporation of stabilizing lipids with high phase transition temperatures tends to decrease drug release (Anderson and Omri 2004; Bochot *et al.*, 1998; Ruel-Gariepy *et al.*, 2002). One of the major challenges of liposomal drug delivery is the rapid uptake of liposomes by the RES. Sterically stabilized liposomes with PEG chains with increased circulation half-life have been developed to decrease contact with blood components, and consequently avoid recognition by the RES system. In clinical studies, the blood circulation half-life of these "Stealth" liposomes was extended from a few hours to 45 h consequently, altering tissue distribution of drugs compared to free drug controls due to their prolonged circulation. pH sensitive liposomes have been formulated to undergo phase change in the acidic environment resulting in a disruption of the lysosomes, and consequent release of the liposome contents into the cytoplasm (Simoes *et al.*, 2004). Immunoliposomes where immunoglobulins are attached to liposomes *via* covalent binding or by hydrophobic insertion to increase their targeting capabilities have also been formulated. Other types of liposomes have been also where ligands such as folate, transferrin mediated liposomes have been utilized to target tumor tissues. More detailed information on recent advancement on the types of liposomes can be found in a review by Torchilin (2005).

An FDA Draft Guidance document for industry on liposome products states that the characterization of physicochemical properties of liposomes is critical to

determine product quality (FDA Draft Guidance, 2002). These tests include determination of morphology, i.e., lamellarity, net charge, volume of entrapment in the vesicles, particle size and size distribution, phase transition temperature, *in vitro* drug release from the liposomes, osmotic properties, and light scattering index. The guidance document states that information on *in vivo* integrity of liposomes should be determined prior to measurement of pharmacokinetic parameters of liposomes. In addition to the information on general pharmacokinetic parameters (i.e., C_{max}, AUC, clearance, volume of distribution, half-life) *in vivo*, comparative mass-balance studies of drug substance and its liposomal formulation were recommended to determine systemic exposure (FDA Draft Guidance, 2002). In order to ensure quality control of the product, chemical stability of liposomes such as phospholipid hydrolysis, nonesterified fatty acid concentration, autooxidation, and drug stability should be identified (Crommelin and Storm, 2003).

Jain *et al.* investigated acyclovir release from multivesicular (MVL) and conventional multilamellar (MLV) vesicles for *in vitro* and *in vivo* studies. They were able to show sustained release in 96 h with MVL liposomes, while MLV's exhibited faster release kinetics in 16 h using dialysis as an *in vitro* release method. Using an *in vivo* rat model, they were able to show sustained plasma levels of drug from MVL up to 32 h, concluding that MVL offered advantages of high drug loading and sustained release with reduced toxicity (Jain *et al.*, 2005).

One of the most commonly used methods to investigate drug release from liposomes is the dialysis method where drug loaded liposomes are placed in dialysis tubes and suspended in a beaker. However, often lack of IVIVC was observed with this method possibly due to violation of sink conditions. Shabbits *et al.* developed an *in vitro* release method using excess amounts of multilamellar vesicles (MLV) as "acceptors" for drug release from "donor" liposomes. They were able to mimic *in vivo* drug release closely using MLV based *in vitro* method which served as a lipid sink (Shabbits *et al.*, 2002). Level A correlation on MVL liposomes was demonstrated by Zhong *et al.* where IVIVC was achieved using plasma as an *in vitro* release medium for drug release (Zhong *et al.*, 2005). However, the use of IVIVC for liposomal products for biowaivers and bioequivalence studies might be difficult since these systems can be very complex. For example, stealth liposomes should remain stable *in vivo*, without any significant release of drug, until uptake into the cells of interest. As expected, such a release profile would be extremely difficult to mimic *in vitro*.

11.7 Emulsions

Emulsions are formed when two or more immiscible liquids with limited mutual solubility are mixed with a high energy input such as *via* ultrasonication, homogenization, or microfluidization. Due to their thermodynamic instability, the use of surfactants is required to improve their stability. Emulsion can be categorized as simple emulsions such as water-in-oil (w/o), or oil-in-water (o/w), or multiple emulsions water-in-oil-in-water (w/o/w) or oil-in-water-in-oil (o/w/o). The most

commonly used clinical application of emulsions is for the delivery of parenteral nutrition for patients who can not absorb nutrients *via* the GI route. These nutrients include vitamins, minerals, amino acids, and electrolytes. Emulsions may also be formulated to deliver drugs with low water solubility, for example propofol formulated in o/w emulsion with soybean oil, glycerol and egg lecithin is currently on the market as a sedative-hypnotic agent (http://www.astrazenecaus.com/pi/diprivan.pdf). Other currently available emulsion products can be found in the Table 11.2.

In vitro release of drugs from emulsion systems can be evaluated using the sample and separate method, dialysis and reversed dialysis methods. Since drug release from emulsions is often relatively rapid, the reversed dialysis is recommended so that sink conditions are not violated.

In vivo pharmacokinetic profiles of drugs in emulsion formulations depends on the blood circulation time, the droplet size of the emulsion, the injection volume and the drug lipophilicity (Kurihara *et al.*, 1996; Takino *et al.*, 1994; Ueda *et al.*, 2001). As described above emulsion formulations also suffer rapid uptake by the RES system. In order to improve blood circulation half-life, Reddy *et al.* investigated pegylation of etoposide emulsion. *In vivo* studies using a rat model, showed that pegylated emulsion exhibited a 5.5 times higher AUC compared to the etoposide commercial formulation. The effect of different oxyetylene moieties varied by size on the o/w emulsion blood circulation time was investigated. It was reported that blood circulation half-life was prolonged from approximately 10 min to 100 min when oxyetylene varied from 10 to 20, respectively. Reduction in the liver uptake was observed with emulsions prepared with 20 and higher oxyetylene moieties compared to those with ten oxyetylene moieties (Ueda *et al.*, 2003).

11.8 Hydrogels, Implants

The advantages of hydrogels as depot formulations are their biocompatibility, water permeability, and injectability (*in situ* forming gels) at the site (i.e., tumor site) (Hoffman, 2001; Peppas *et al.*, 2000). One disadvantage of hydrogels is that the drug release rate may not be manipulated, for example: fast release rate of hydrophilic drugs occurs from the hydrogels due to the hydrophilic environment within the hydrogel. Therefore, two phase systems were developed where delivery vehicles (liposomes or microspheres) were entrapped in the hydrogels to control drug release kinetics (Galeska *et al.*, 2005; Moussy *et al.*, 2003; Patil *et al.*, 2004). Patil *et al*. (2004) were able to achieve *in vitro* and *in vivo* controlled release of dexamethasone from microspheres entrapped in a polyvinyl alcohol hydrogel and a linear *in vitro–in vivo* correlation of release rates. Lalloo *et al.* (2006) demonstrated *in vitro* and *in vivo* controlled release of chemotherapeutic topotecan from two phase systems where drug containing liposomes were entrapped in hydrogels. Longer tumor suppression was achieved using with this approach compared to drug alone.

A linear IVIVC was established for methadone release from implants (Negrin *et al.*, 2001). In this study drug release *in vivo* was calculated from the amount of drug remaining inside the implant. However, when *in vivo* methadone release was estimated by deconvolution from serum levels, deviations from linearity occurred at later time points. The authors confirmed the role of possible metabolic induction in the underestimation of *in vivo* release as a consequence of increased methadone clearance with time. Therefore, it should be noted that estimation of *in vivo* release by deconvolution might not be applicable when the drug absorption and disposition function is not linear and not constant with time (Negrin *et al.*, 2004).

11.9 Dendrimers

Dendrimers are synthetic highly branched polymers with a central core with sizes in the nanometer range. The structure and branched topologies of dendrimers resembles a branched tree hence the name is derived from the Greek name *dendra* (meaning tree, tree-like structure). Dendrimers can be categorized based on the number of the branches they possess which are called generations (G-1, G-2, G3, etc.). The molecular weight, chemical composition and size of the dendrimers can be tightly controlled during synthesis of these polymers. Most commonly used dendrimers are based on polyamidoamines (PAMAM), polyamines, and polyesters (Frechet and Tomalia, 2002; Newkome *et al.*, 2001). Dendrimers are ideal candidates as drug/gene delivery carriers, biological imaging agent carriers, and as scaffolds in tissue engineering due to their uniform size, monodispersity, water solubility, modifiable surface characteristics and high drug loading efficiencies (Kobayashi and Brechbiel, 2004; Kukowska-Latallo *et al.*, 1996; Patri *et al.*, 2002). In addition, the surface charge of these polymers can be manipulated to increase biocompatibility and decrease toxicity. For example, it has been shown that the cytotoxicity of cationic PAMAM dendrimers decreased when the surface charge was modified with the addition of lauroyl and PEG chains (Jevprasesphant *et al.*, 2003). It has also been shown that dendrimers can be used as a multifunctional delivery platform loaded with therapeutics, targeting and imaging agents. PAMAM dendrimers loaded with methotrexate (MTX) as a chemotherapeutic, folate as a targeting agent, and fluorescein as an imaging agent accumulated preferentially approximately five times higher than the control in a mouse model with subcutaneous tumors (Kukowska-Latallo *et al.*, 2005). Drugs can be either physically entrapped or conjugated with the dendrimer. However, it has been shown that MTX was readily released in saline when physically entrapped in dendrimers. This was attributed to weak interaction between MTX and the dendrimer when inter molecular forces are neutralized in the PBS solution. However, MTX was retained in the dendrimer and did not exhibit any premature release when conjugated to the dendrimer (Patri *et al.*, 2005). Drug release from the dendrimers can be controlled by change in pH, for example ester terminated half generation PAMAM dendrimers did not release any drugs at pH 7.0. However, drug release occurred at pH 2.0 when internal tertiary amines were protonated (Twyman *et al.*, 1999). In chemotherapy, this pH responsive release mechanism is desired since

drug release occurs only in the acidic microenvironment of the tumor tissue not in the systemic circulation. Another example of controlled drug release from dendrimers was the sustained release of indomethacin from dentritic unimolecular micelles (Liu *et al.*, 2000).

Similar to other MR release products mentioned above, different strategies such as modification of the dendrimer surface with polyethyleneoxide, PEG chains, have been successfully applied to decrease their RES uptake (Gillies and Frechet, 2002; Kim *et al.*, 2004; Malik *et al.*, 2000; Wang *et al.*, 2005; Yang and Lopina, 2006). Neutral, generation four (G4) polyester dendrimers did not accumulate in any organ preferentially and they exhibit rapid renal clearance. It was noted that the low molecular weight and compact structure of the neutral G4 dendrimers could pass glomerular filtration (PadillaDeJesus *et al.*, 2002). In a follow-up study, it was shown that highly branched dendrimers (Generation 3) exhibited greater bioavailability and lower renal clearance than that of the compact dendrimers (Generation 2) where the molecular weights of these dendrimers were approximately the same (Gillies *et al.*, 2005). The potential value of dendrimers as a delivery vehicle is promising since biodistribution and pharmacokinetic properties can be manipulated by changing the dendrimer size and conformation (Lee *et al.*, 2005).

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