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Review

In vitro and *in vivo* considerations associated with parenteral sustained release products: A review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting ☆

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Contents

1. Introduction

The evolving sophistication of human and veterinary therapeutic objectives necessitates the development of innovative drug delivery systems. Often formulated as modified release (MR) parenteral dosage forms, these complex products can be engineered to maintain such characteristics as zero order or pulsatile drug release for a duration of weeks, months or even years. When functioning as intended, these products are invaluable for improving user compliance and for addressing the therapeutic needs of the human [1,2] or veterinary [3–5] patient. MR parenteral drug products are available in several dosage forms, including microspheres, liposomes, gels, suspensions, implants, lipophilic solutions, and drug eluting stents.

The complex nature of MR parenteral products necessitates the use of *in vitro* release testing as an indicator of product performance. However, the development of *in vitro* release test methods that predict the *in vivo* performance of these products is encumbered by the lack of standard methods, the need to accelerate the *in vitro* release rate

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relative to that occurring *in vivo*, instability of the system (or drug), and failure to accurately reproduce the *in vivo* environment. *In vitro* tests are also generally unable to accurately predict the effect of host–product interactions that can negatively influence product performance or that are responsible for the targeted and/or prolonged duration of action. The summary reports from two previous workshops on *Assuring Quality and Performance of Sustained and Controlled Release Parenterals* [6,7] emphasized the need for guidance on *in vitro* release testing apparatus and methodologies suitable for MR parenterals.

Understanding the factors influencing drug release, both from an *in vivo* and an *in vitro* perspective, facilitates the development of meaningful *in vitro* release tests and performance specifications. To allow for discussion of these aspects in a public forum, the Controlled Release Society (CRS) hosted a 2007 Educational Workshop entitled: Sustained Release Parenteral Products: *In Vitro* and *In Vivo* and Considerations. This workshop provided the opportunity to engage pharmaceutical scientists and pharmacologists in discussions regarding the critical variables impacting the development of novel parenteral sustained or MR formulations. The speakers included scientists representing government, academia and industry.

The goals of this interactive session were:

1. To provide a forum in which pharmaceutical scientists (industrial, academic and regulatory) could discuss current issues, and future objectives of MR parenterals used in human and veterinary medicine.
2. To examine the various types of MR parenteral formulations from the perspective of:
   - The critical manufacturing variables.
   - The relationship between the mechanism of release and formulation variables as they influence the *in vitro* test conditions, *in vivo* product performance and product release specifications.
   - Challenges associated with the development of the *in vitro* test methods.
   - The critical biopharmaceutic issues, (e.g., physiological variables, bioavailability).
3. To address and discuss the possibility of *in vitro–in vivo* correlation (IVIVC) and Chemistry, Manufacturing and Controls (CMC) considerations.
4. To enhance the exchange of information to foster future drug development.

Speakers and topics included:

I. Developing *in vitro* test methods and setting *in vitro* release specification
   - *Human Parenteral Sustained Release Formulations: Examples and Regulatory Challenges*
     Mansoor Khan, FDA/CDER, U.S.A.
   - *Veterinary Parenteral Sustained Release Formulations: Examples and Regulatory Challenges*
     Mai Huynh, FDA/CVM, U.S.A.
   - *Microspheres: Developing "In Vitro" Test Methods and Setting "In Vitro" Release Specifications*
     Diane Burgess, University of Connecticut, U.S.A.
   - *Subcutaneous Implants: Developing In Vitro Test Methods and Setting In Vitro Specifications*
     Jeremy Wright, Durect Corporation, U.S.A.
   - *Liposomes: Developing In Vitro Test Methods and Setting In Vitro Release Specifications*
     Diane Burgess, University of Connecticut, U.S.A.
   - *Lipophilic Solutions and Suspensions: Developing In Vitro Test Methods and Setting In Vitro Release Specifications*
     Susan Weng Larsen, University of Copenhagen, Denmark
   - *"In Vitro–In Vivo" Correlations (IVIVCs): Human Examples*
     Jaymin C. Shah, Pfizer, U.S.A.

II. The impact of host physiology on *in vivo* product performance
   - *Physiological Variables Influencing Product Performance*
     Natalie Medlicot, University of Otago, New Zealand
   - *Factors Influencing "In Vivo" Drug Release: Microspheres, Nanoparticles and Implants*
     Uday Kompella, University of Nebraska, U.S.A.
   - *Unique "In Vivo" Challenges with Parenteral Liposomal Preparations: Describing Pharmacokinetic Behavior*
     Daryl C. Drummond, Hermes Biosciences, Inc., U.S.A.
   - *Human Safety Considerations*
     Patrick Marroum, FDA/CDER, U.S.A.
   - *Veterinary Safety Considerations*
     Marilyn Martinez, FDA/CVM, U.S.A.

This review article is an effort to capture the wealth of information presented by the speakers and from subsequent audience participation at that workshop. Presenters have been asked to review and comment on the contents of this manuscript. We express our gratitude for their cooperation.

2. Setting specifications

When establishing specifications for MR parenteral dosage forms, it is important to appreciate the variables that can influence drug absorption. To begin with, it is helpful to consider these factors as they relate to simple aqueous solutions and suspensions (Table 1) [8–10].

While the majority of small molecules are absorbed through the vasculature, lymphatic absorption becomes increasingly important as molecular weight increases. Therefore, proteins larger than 16 to 20 kDa are generally thought to be absorbed primarily through the lymphatics [11].

Understanding the variables that can influence *in vivo* product performance helps in determining critical parameters of the MR parenteral dosage form that may become the target of finished product tests and specification development. Product quality issues relating to setting specifications of MR parenteral dosage forms include:

- Particle size
- *In vitro* release characteristics
- Residual solvents
- Osmolarity
- Product sterility
- Product stability
- Biomaterial characteristics

Table 1
Factors influencing the absorption of drugs from solutions and suspensions [8]

Aqueous solution	Aqueous suspension
The solution spreads rapidly, increasing the surface area for absorption.	Absorption is inversely related to particle size and density (due to decreased spreading of material).
Dissolved molecules may diffuse more slowly than the aqueous solvent. In part, separation of solute and solvent is promoted by the tissue ground substance that can act as a molecular filter [9,10]. This results in local changes in drug concentration.	Absorption is proportional to *in vivo* drug solubility.
Solute absorption rate is proportional to $1/V_{injection}$, the concentration of solute at the absorption site, and the surface area for absorption.	For many formulations, absorption rate constant is inversely related to injection volume and initial concentration. This is suggested to be due, at least in part, to particle agglomeration.
Aqueous solvent may be absorbed more rapidly than the solute, further increasing local drug concentrations.	
High tonicity can decrease drug absorption rate.	

Table 2
Comparison of median particle sizes measurements [14]

Size fraction (μ)	Laser diffraction	Image analysis	Light obscuration
20–32	38	52	48
32–45	50	63	64
45–63	66	76	85
63–90	86	95	105
90–150	114	105	134
150–212	87	99	108

- Crystalline/polymorphic form of the active pharmaceutical ingredient
- Polymer molecular weights
- Residual moisture
- Drug entrapment efficiency
- Bioburden (including endotoxin levels, heavy metals etc.).

ICH Q6A [12] and VICH GL39 [13] define specifications as "a list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. Specifications are critical quality standards that are proposed and justified by the manufacturer, and are subsequently approved by regulatory authorities as conditions of approval". "Conformance to specifications" means that the drug substance and/or drug product, when tested according to listed analytical procedures, will meet the listed acceptance criteria.

When setting specifications, the two fundamental issues to consider are defining the critical attributes and establishing criteria for accurately controlling that attribute. For example, if particle size is identified as a critical quality attribute, then the establishment of process controls needs to begin by identifying/verifying/validating an optimal method for particle size assessment. That being said, such an undertaking poses its own set of challenges because particle size measurements can vary as a function of the method employed. An illustration of this point is shown in Table 2 where the median diameter (d_{50}) estimated using three different methods of particle size measurement was compared as a function of particle size fraction [14]. As seen in this table, markedly different median diameter values were obtained across methods, some predicting values well outside the limit of the actual range of particle sizes for that fraction.

In this example, the pivotal questions to address when selecting a suitable analyzer method include:

- Is the method widely used/well understood?
- Are standards available to validate the method and to test its robustness?
- What are the limitations of the method with respect to size, shape, agglomeration, cohesiveness, flowability, brittleness, availability, phase of development etc.?
- Will the equipment be available for the life of the product?

Once the particle size analyzer has been selected, specifications can be established. As a result, it is expected that the same method/instrument be used throughout the life cycle of the product, unless comparison data are available to support the change (e.g., to a new method or specifications). Such an approach is applicable to any analytical method.

3. Sterilization as a critical manufacturing variable

Besides the unique steps that may vary from one dosage form to another, sterility assurance is a common aspect to all injectables since they are required to be sterile. While aseptic processing may be appropriate for some drug/dosage forms, it may be inappropriate for others. Therefore, alternative means of sterilization have been explored and used, such as terminal sterilization by moist heat or irradiation.

The impact of the sterilization procedure and the specifications associated with that method selection need to be carefully considered. Gamma irradiation can lead to product degradation. It has been shown to damage the phospholipids in liposome products, lead to the loss of active pharmaceutical ingredient, and alter the chemical properties of excipients [15]. In addition, gamma irradiation-induced polymer degradation can affect both polymer molecular weight and glass transition temperature. These changes can affect the release profile of the product. To better understand these effects, researchers typically study the chemical and physical characteristics of the product and its excipients before and after irradiation. Including an overage of the active pharmaceutical ingredient can compensate for loss due to the irradiation process. However, the overage needs to be carefully considered and justified.

4. Development of *in vitro* release tests

For MR release dosage forms, it is often necessary to use an *in vitro* method of release testing that exceeds the *in vivo* rate of drug release. Since these dosage forms are typically designed to release their contents over periods of weeks, months or even years, it becomes impractical to wait for a real-time test for batch release of product. Therefore, accelerated methods are often developed to assist in batch release of product. Accelerated tests, by their nature, (e.g. elevated temperature or use of solvents) can change not only the rate of drug release but also the mechanism of release. Therefore, it is very important to understand the accelerated method and how it may affect the drug release mechanism. In some cases there may be a good correlation between accelerated release profiles of different formulations and their real-time profiles, however this may not always be the case. The reader is referred to manuscripts by Zolnik et al. that detail studies of the affect of accelerated temperature and pH on the release profiles of different MR microsphere formulations [16–18]. It is possible that the mechanism of release can change such that even the rank order of the release profiles of different formulations change. Consequently care needs to be taken in selecting an accelerated release method.

The purpose of the test should also be considered. For example, for tests intended to support an IVIVC, the release profile from an accelerated test should correlate with the *in vivo* release profile. Where it is not possible to achieve such a correlation with an accelerated release test, such a test may still be useful for batch release of the product. However, the development of an additional real-time test will still be needed if the intent is to develop an *in vitro* test that is predictive of *in vivo* product performance. Accordingly, the purpose for developing an *in vitro* drug release test needs to be considered. Examples of potential purposes include:

- The quality control for batch release;
- An assessment of the impact of manufacturing process changes;
- The substantiation of label claims;
- An evaluation of the potential for dose dumping;
- An assessment of *in vivo* stability;
- The prediction of *in vivo* performance and
- The establishment of an IVIVC.

Although the design of the *in vitro* test may vary as a function of the test objective (e.g., batch release of product would usually necessitate the development of an accelerated test), it is important from a scientific perspective that all tests are designed with bio-relevance as the ultimate goal. While this can be a difficult objective to achieve [19–21], efforts to establish a bio-relevant procedure can greatly facilitate the development of predictive specifications for product release and stability.

Optimally, the *in vitro* method will allow for the characterization of at least 80% of the drug load. However, this kind of target often necessitates

Table 3
Examples of oil solutions and suspensions

Type	Drug/prodrug	Vehicle
Oily solutions	Testosterone enanthate	Castor oil
	Nandrolone decanoate	Sesame oil
	Fluphenazine enanthate	Sesame oil
	Zuclopenthixol decanoate	Medium chain triglycerides
Oily suspensions	Penicillin G procaine	Vegetable oil
	Bovine somatotropin	Sesame oil
	Ceftiofur	Cottonseed oil

the use of accelerated test conditions to minimize drug and drug product degradation. Since most tests will inevitably extend over several days, the selection of the medium should be chosen with care to ensure minimal evaporation and maximum stability of the drug. The inclusion of an appropriate preservative may also be necessary.

Compared to immediate release dosage forms, MR parenteral dosage forms have additional quality and performance considerations that need to be taken into account when evaluating product release and defining stability specifications [22]. In particular, a product administered to a patient at expiry should be safe and effective throughout the time it is inside the body.

Developing an *in vitro* dissolution or drug release test that correlates with *in vivo* product performance allows for the *in vitro* test method to both serve as a quality control tool and as an indicator of *in vivo* performance [20]. However, it is important to recognize that when dealing with MR systems, it is the mechanism of release that should dictate the science of the *in vitro* test method which, in turn, determines the suitability of the results. In other words, a test method that works for one mechanism of release will not necessarily work for a system operating with a different release mechanism. For that reason, it is important to avoid having standard methods that are "set in stone".

In the spirit of providing a starting point to consider, it was interesting to consider some of the test methods that have been successfully employed for a range of MR products. For example, success has been reported with the use of a modified rotating paddle for suspensions, Franz cell diffusion system for gels, flow-through cell for implants, and floatable dialysis bag for microspheres or nanoparticles [20]. Correlation has also been reported with USP apparatus 4 for microspheres [18]. A good correlation has also been reported with a static medium for the DUROS® Viadur® leuprolide implant [23].

Important factors to consider in the selection of an apparatus include its agitation characteristics and flow rate. Regarding the choice of medium, to the extent possible, the medium should mimic the physiological conditions of the target animal [24]. While sink condition is a factor to consider, this may not always be possible to achieve for some of the complex formulations. In addition, the apparatus and the method need to be simple and practical for use as a quality control method. However, the method also needs to allow for discrimination between formulations, manufacturing process conditions, and stability variants that can affect the *in vivo* release rate of the drug product.

In addition to the aforementioned product considerations, variables influencing the biological responses can be regulated through the development of manufacturing specifications. These include:

- Drug content uniformity
- Drug stability/degradants (not only during shelf life but also while implanted)
- Endotoxins.

A summary of considerations associated with the development of *in vitro* drug release tests for selected parenteral controlled release dosage forms, as discussed during the workshop, are provided below.

4.1. Subcutaneous implants

Several different long acting subcutaneous implants are available in the human and veterinary market. These can be classified into the following groups:

- Compressed pellets: e.g., Finaplix-S® (trenbolone acetate) and Synovex®-H (estradiol benzoate + testosterone);
- Diffusional matrix: e.g., Compudose® (estradiol);
- Diffusional — membrane controlled: e.g., Norplant®;
- Bioerodible matrix: e.g., Zoladex® (goserelin acetate), DURIN™ Membryte™ (leuprolide acetate) and Gliadel® wafer (carmustine); and
- Osmotic pump/Duros®: e.g., Viadur® (leuprolode acetate) and Chronogesic® (sufentanil).

Tissue reactions at the site of parenteral drug administration include acute inflammation, chronic inflammation, granulation, foreign body reaction and fibrosis [25]. The formation of fibrous capsules around implanted materials can compromise drug release [26]. Therefore, biological factors to consider when developing an *in vitro* release test for a subcutaneous implant include: (i) the subcutaneous environment, its configuration, foreign body response such as immune system cells and encapsulation; (ii) the *in vivo* release mechanism of implant (e.g., diffusion, erosion or osmosis); (iii) *in vivo* uptake of the drug and (iv) the complex interaction between the host and the implant.

The biological environment surrounding the subcutaneous implant is not static, and it may not be possible to capture some of the biologically relevant reactions through the use of *in vitro* systems, such as inflammation with consequent cellular recruitment and fibrosis capsule formation. Another example is the possibility of *in vivo* implant fragmentation, leading to an increase in the surface area available for drug release, higher systemic drug concentration, potential safety concerns, and a failure to maintain *in vivo* therapeutic drug concentrations for the intended duration of therapy.

Release rate from implants (and from microspheres) can be influenced by design variables and excipient variables such as polymer molecular weight, copolymer ratio and water content. Critical manufacturing variables include drug loading, length, thickness and particle size that can also influence the rate and extent of drug release. Likewise, changes in drying speed can influence the presence or absence of burst release, where too rapid drying can lead to co-transport of drug with solvent to the surface of the implant, leading to segregation and non-uniform drug release patterns [27].

4.2. Lipophilic solutions and suspensions

Examples of marketed oil depot solutions and suspensions are given in Table 3.

Assessment of drug release rates from oil solutions and suspensions has been successfully achieved *in vitro* using a rotating dialysis cell model [28–31]. This *in vitro* model may constitute a valuable tool for describing the effect of drug and formulation characteristics on drug

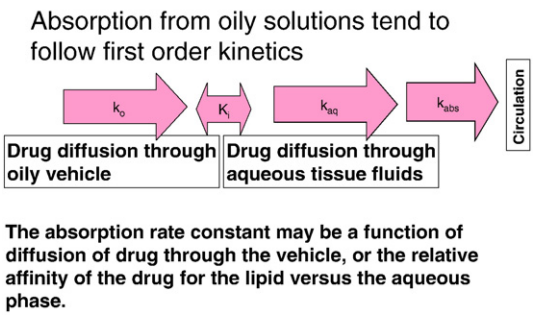

Fig. 1. Bioavailability of oily solutions.

Table 4
Factors influencing the absorption of lipophilic solutions and suspensions [36]

Lipophilic solution or lipophilic suspension	
Oil:water partition coefficient	The greater the affinity for the lipoid phase, the slower the absorption process.
Diffusivity of drug in tissue fluids	For lipophilic solutions, this is not a rate-limiting factor.
Diffusivity of drug in the vehicle	Generally, this is a rate-limiting event only n the case of in situ forming gels.
Initial drug concentration	Unlike aqueous suspensions, the initial concentration has minimal effect on the absorption rate constant.
Surface area	The greater the surface area, the faster the absorption rate constant.
Injection volume	Injection volume negatively affects the absorption rate constant.
Clearance of oily vehicle	Highly lipophilic drugs may be released concurrently with the disappearance of the oil vehicle from the injection site.
Release from oil suspension	The transport of solid drug particles to the oil:water interface or particle dissolution in the oil phase may influence the rate of drug absorption.

release rate from oil solutions and to describe the *in vitro* release and transport processes in a quantitative manner. The rotating dialysis cell model offers the advantages of reproducible results and fast distribution and dissolution processes (*i.e.*, it is a rapid test). In some cases, it has been shown to produce estimates of *in vitro* percent drug release profiles that are highly correlated with *in vivo* release kinetics [32]. Commercially available Float A Lyzer® dialysis tubes can also be used as an alternative *in vitro* model operating at much less intensive stirring conditions to assess drug release from oil solutions and suspensions [28,31,33] as well as from biodegradable microspheres [32]. In general, dialysis membrane-based models are considered feasible for the study of drug release from depots administered in small aqueous body compartments where the drug is released under non-sink conditions [34,35].

The sustained release characteristics of lipophilic solutions are dependent upon the relative affinity of the drug for the oily vehicle versus the aqueous environment of the tissue [36,37]. This is diagrammatically represented in Fig. 1. This relative affinity determines the speed at which the drug partitions out of the oily matrix and into the tissue fluids, and is described by the oil/water partition rate coefficient, K_i. Only in the case of gels does the diffusion of drug through the vehicle (as described by the diffusion rate coefficient, k_o) serves as the rate-limiting factor in drug absorption, where k_{abs} represents the rate constant for drug absorption. Furthermore, assuming sink conditions, the rate of diffusion through the aqueous medium, as described by the diffusion rate coefficient k_{aq}, is not the rate-limiting step in drug absorption. A summary of the variables that can influence the parenteral absorption of lipophilic solutions and suspensions is provided in Table 4 [36].

With the exception of in situ forming gels, absorption rate is governed predominantly by the distribution coefficient (K_i) between the oily vehicle and the aqueous fluids, and has little dependence on vehicle viscosity [36]. In these cases, absorption can be described by the equation:

$$k_{abs} = Ak_{aq}(V_0K_i)$$

where A = surface area, and V_0 = injection volume. In this case, K_i is the rate-limiting factor in drug absorption and k_{abs} is the absorption rate constant.

Conversely, in the case of in situ forming gels, absorption rate can be described as follows:

$$k_{abs} = AK_0/V_0.$$

4.3. Microspheres

A variety of *in vitro* testing methods have been applied to microspheres, such as membrane diffusion, as well as the continuous flow method (USP apparatus 4). The continuous flow method was originally designed for MR oral products but has recently been shown to be an appropriate for testing for MR parenteral microspheres. The USP apparatus 4 flow-through cell has been modified by the addition of glass beads that help to prevent microsphere aggregation and facilitate laminar flow of the media throughout the cell [38]. This ensures a uniform exposure of the microspheres to the media. Although the USP apparatus 4 appears to be an appropriate method for examining the *in vitro* release characteristics of MR microsphere products, there may be some cases where the use of other apparatus could be justified.

Media selection for microsphere products can depend upon microsphere composition, drug solubility and the purpose of the test (accelerated, IVIVC etc.). The temperature, pH and ionic strength may be varied and solvents, surfactants and other substances may be added [16–18]. The volume of the media may also be altered to accommodate drug solubility and dose, as well as to simulate the *in vivo* environment. However, similar to implants, host responses to foreign material can alter product release rate.

In some cases, polylactic glycolic acid (PLGA) microspheres undergo agglomeration, followed by surface changes [39,40]. This can be avoided in *in vitro* testing by using the modified USP apparatus 4 method as described above [18]. The nature of these *in vivo* changes can determine product release characteristics. In this regard, there are two types of degradation that can occur:

- *Heterogenous*: The polymer degrades more rapidly than rate at which water enters into the polymer. Degradation occurs primarily at the surface (erosion). This occurs with polymers containing highly reactive groups and for relatively small microspheres or nanoparticles and leads to a relatively controlled drug release.
- *Homogenous*: Bulk eroding polymers degrade more slowly and imbition of water into the system is faster than polymer degradation. The device is wetted and chain cleavage occurs throughout the system. This can lead to irregular drug release. Ultimately, the resulting porous mass can have an inward collapse leading to drug entrapment (especially proteins).

Bulk eroding PLGA polymers lead to autocatalysis since the oligomeric units that are generated cause a local pH drop, which in turn catalyzes further hydrolysis. This phenomenon is more likely to occur with larger, as compared to smaller, microspheres due to the acid accumulation in response to the larger diffusional distances. For smaller particles, the micro-environment can be better maintained since buffers can diffuse into microspheres and H+ can diffuse out relatively rapidly [41,42].

There is also the possibility of chiral-specific interactions. Wang et al., showed that excipients with chiral centers, such as DL-PLG, can interact with chiral drugs, such as ketoprofen, as the implant/microsphere begins to degrade [43]. While fast heterogeneous degradation (surface erosion) will not result in stereospecific release characteristics, a chiral interaction can occur with the slow, homogenous degradation process. As a result, the transient production of chiral domains can lead to a delayed onset of stereospecific drug release.

Microsphere particle size and size distribution also have an impact on content uniformity and syringeability, which ultimately affect the bioavailability of the drug product.

4.4. Liposomes

Liposomal preparations tend to be biodegradable and non-immunogenic. Although liposomes do not reduce the acute toxicity of the encapsulated agent, it does alter the overall toxicity profile in a favorable manner. Encapsulation can also make a significant difference in the types of toxicity responses observed. For example, the dose limiting toxicity of rapid release doxorubicin is myelosuppression, while the toxicity associated with the slow release formulation is mucositis and hand and foot syndrome. For both rapid and slow

releasing formulations, cardiotoxicity is a concern. However, when administered as a liposomal preparation, doxorubicin remains in the circulation for a prolonged duration, and systemic toxicity is minimized since the liposomes are passively targeted to the tumor site, thus reducing drug concentrations in the rest of the body [44,45].

The duration of drug exposure from these preparations depends upon the clearance of the lipidic nanocarrier, the rate of release of the drug from the carrier system, and the clearance of the free (unencapsulated) agent once released from the liposome. Not all liposomes are equivalent, both in terms of the stability of the liposome and the ability of the drug to diffuse through the liposomal membrane. Factors influencing the pharmacokinetics of liposomal nanocarrier systems include:

- Surface charge: extremes of charge tend to increase clearance and sterically hindered anionic lipids or low concentrations of certain anionic lipids can prolong the residence of the liposome in the circulation;
- Size: size is positively correlated with an increase in drug clearance;
- Lipid packaging: highly ordered gel phase lipids with cholesterol tend to increase clearance rate;
- Pegylation: decreases clearance;
- Dose: conventional liposomes tend to be associated with dose_dependent kinetics;
- Encapsulated agent:
  - Not all therapeutic agents can appropriately be encapsulated:
    - Hydrophobic drugs tend to partition in the membrane. In these cases, liposomes are used primarily for drug solubilization;
    - Highly charged/hydrophilic drugs are generally passively encapsulated, resulting in typically low loading efficiencies. Low drug to lipid ratios tend to result in low product stability. Proteins may be more suitable as polymer formulations;
    - Amphipathic drugs (such as weak amines) are actively loaded at high concentrations, forming very stable formulations. While weak acids are also actively loaded, there remains some question with regard to the chemical stability of these liposomes;
    - Polyanionic agents (such as nucleic acids) can form complexes with cationic lipids. While this technology appears to be promising, it is still in its infancy;
  - High drug/lipid ratios help to increase the intra_liposomal drug concentrations, often in excess of their aqueous solubility limits. However, excessively high ratios may dissipate gradients that help to maintain the encapsulations;
- Targeting ligand: Optimally, the ligand should have no influence on the pharmacokinetics of the drug unless the target is vascularly accessible. It generally takes approximately 24 h to have liposomes accumulate in solid tumors.

There are several novel liposomal delivery systems that are currently under investigation. One of these systems involves drug co-encapsulation for the purpose of maintaining optimal drug ratios for a prolonged duration. Currently, however, difficulties in formulating these systems have been encountered, and an adequate delivery of one drug may be met with challenges in maintaining delivery rate for the second compound. Consequently, these co-encapsulated formulations may not maintain the optimal drug ratio over time [46].

Liposomes are also being developed to provide a triggered release upon reaching its target site. Examples of strategies currently under investigation include ligand targeted liposomes to internalize epitopes [47,48], pH-sensitive liposomes [40,49], thermosensitivity liposomes [50], redox-sensitive liposomes [40], "SMART" systems [51,52], and ultrasound-sensitive liposomes [53].

It is feasible that a microdialysis method can be used to screen liposomal formulations for stability in the presence of human plasma. However, *in vitro* stability does not insure *in vivo* stability. Moreover, as these highly sophisticated targeted delivery systems are under development, it will be challenging at best to devise an *in vitro* test condition that can adequately reproduce the fluctuations in *in vivo* conditions that ultimately affect drug release.

Performance testing of liposomal products is more complex than microspheres. There are several different types of liposomes, as discussed above, from conventional (immediate release) liposomes to controlled release, "stealth" and immuno-liposomes. Liposomes can also be targeted to specific areas in the body depending on their size. These different liposomes have different release characteristics. For example "stealth" liposomes are designed to be stable with no or very little release until uptake into cells, whereas immediate release liposomes are designed to release their contents immediately following injection. Consequently, *in vitro* testing may need to be adjusted to reflect the nature of the liposomal formulation. As for microspheres, there currently is no standard *in vitro* release method. For "stealth" liposomes in particular the development of an *in vitro* test is challenging. *In vitro* tests that have been used for liposome products include: conventional USP methods, sample and separate methods, membrane dialysis, flow-through methods; and agar gel methods.

The dialysis sac method is most commonly used for liposome products. However, a variation on the dialysis sac method, the reversed dialysis sac method, has been shown to be more appropriate for rapidly releasing systems such as liposomes and emulsions due to a violation of sink conditions that can occur in the dialysis sac method for rapidly releasing dispersed system formulations [54]. This violation of sink conditions occurs as a result of the relatively rapid release from the dosage form into the donor chamber (dialysis sac), the limited volume in the dialysis sac, and the constraint of diffusion across the dialysis membrane. In the reversed dialysis method, the formulation is placed into the bulk phase, rather than within the dialysis sacs — thus considerably enhancing the volume in the donor phase. The dialysis sacs then become the receiver chamber and each sac may be used as a single time point. Slow release from liposome formulations has been reported utilizing a conventional dialysis sac method [55], whereas relatively rapid release from a similar liposome formulation has been reported when a reversed dialysis method was used [56]. Different *in vitro* release methods may be appropriate for different types of liposomes and for liposomes delivered via different routes of administration. Methods utilizing *in vitro* cell culture may be appropriate to assess performance and stability change in the presence of biological cells.

5. Excipient considerations

As with any novel chemical entity, there are potential safety concerns associated with novel and unapproved excipients, residual solvents, impurities and degradation products, and solubilizing vehicles. Therefore, a guidance on the kinds of information needed to support the human use of new drug excipients was published by the U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) in May 2005 [57].

Within the guidance, the term "new excipient" is defined as any inactive ingredient(s) that is intentionally added to therapeutic and diagnostic products, but that: (1) CDER believes to not be intended to exert therapeutic effects at the intended dosage, although they may act to improve product delivery (e.g., enhance absorption or control release of the drug substance); and (2) is not fully qualified by existing safety data with respect to the currently proposed level of exposure, duration of exposure, or route of administration. For any new excipient, a battery of safety tests is needed to support product registration. The kinds of tests that are recommended depend upon whether the excipient will be used in a product that is intended for short (*i.e.*, less than 2 weeks), intermediate (*i.e.*, labeled for use for more than 2 weeks but less than 3 months), or long term exposure (*i.e.*, products intended for clinical use of a duration in excess of 3 months).

6. Safety assessment and *in vitro* testing of MR parenteral dosage forms in veterinary medicine

In veterinary medicine, patient safety of proposed drug products is evaluated by conducting target animal safety (TAS) studies and from the data collected in one or more adequate and well-controlled studies providing substantial evidence of effectiveness. TAS studies are generally conducted in the intended animal species, at exaggerated doses and over a prolonged duration of administration [58].

When drug products are administered to food-producing animals, the human food safety of drug residues in animal-derived tissues needs to be addressed. Prolonged release characteristics need to be factored into the establishment of a time when the edible tissues are safe for human consumption. In this regard, very low concentrations that may have negligible impact on target animal safety and effectiveness could greatly influence the duration of time that animals receiving the drug need to be retained before entering the human food chain. There are several CVM guidance documents associated with the evaluation of human food safety, including the Food and Drug Administration, Center for Veterinary Medicine (FDA/CVM) Guidance #3 (General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals) [59], Guidance # 159 (Guidance for Industry: Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI — VICH GL-36, Final Guidance) [60], and Guidance #160 (Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Repeat-Dose (Chronic) Toxicity Testing VICH GL-37) [61].

New animal drug applications for modified release products must contain data to demonstrate that the dosage form will release the active ingredient(s) at a safe and effective rate and that these release characteristics will be maintained until the expiration date of the drug [21 CFR §500.26(d)]. In this respect, the drug product should be formulated and manufactured by a process that consistently provides a product meeting quality attributes that are the same or similar to the product demonstrated to be safe and effective in the target animal safety and effectiveness studies. It should be noted that currently, veterinary parenteral dosage forms are typically subject to standard testing and specifications as defined in the USP.

7. Developing *in vitro–in vivo* correlations

Although it was recognized by the workshop participants that real-time data for drug release is essential to correlate to *in vivo* bioavailability, it was suggested that accelerated testing can be explored so that the *in vitro* method can be used for quality control purposes. To this end, a relationship needs to be established between accelerated and real-time data. In addition, the conditions that are altered to facilitate accelerated testing need to be carefully examined to maintain the bio-relevance aspect of the test.

Once an *in vitro* release method is established it needs to be tested with appropriate formulation variants that present with differing *in vivo* performance characteristics. If the *in vitro* test is not discriminatory, then appropriate modifications to the test conditions are needed. Furthermore, care should be taken when selecting formulation variants to avoid altering the mechanism of release. If the mechanism of release is changed, it will not be possible to use these formulations to establish an IVIVC [17].

Accelerated testing will often result in a change in the mechanism of release. Nevertheless, accelerated conditions can still serve as a discriminatory tool so long as all formulations experience similar changes and continue to exhibit performance characteristics that can be differentiated from each other. In some cases, a correlation between *in vivo* data and accelerated *in vitro* data may be obtained, regardless of a change in the mechanism of release. However, there are numerous other situations where the use of accelerated test conditions may be problematic. For example, some MR dosage forms are associated with multiphasic release characteristics, such as an initial burst release followed by a secondary release phase. It is often impossible to separate these different phases in an accelerated test. For that reason, a separate "real-time" test is often needed to characterize the initial burst phase. The initial burst release phase is usually diffusion controlled, whereas the later phases tend to be controlled by erosion and diffusion. In this regard, elevated temperatures can often be used to predict release under "real-time" conditions [17]. IVIVC correlations have also been established for microspheres post-burst release [18].

In some cases (e.g., ophthalmic drug delivery systems), a biological barrier presents an additional challenge that may not be adequately described *in vitro*. For example, nano- and micro-particles can be injected perivascularly to promote sustained retinal drug delivery [62–64]. In contrast to periocular particulate systems, intravitreal injections can lead to the aggregation of particles, thereby obstructing vision. The ability for particles to be retained in the subconjunctival region appears to be related to particle size whereby particles of approximately 200 nm or greater are necessary to support sustained drug delivery [64]. However, it is unlikely that this size effect can be well defined through the use of *in vitro* test procedures. Other example where the physiology of the eye itself alters drug release includes an intravitreal implant in rabbits where it is the vitreous fluid that retards the drug release [65]. In the case of an episcleral betamethasone-loaded polyvinyl alcohol (PVA) implant coated with 5% ethylene vinyl acetate (EVA), the drug release *in vivo* was faster than that seen *in vitro* [66]. For a polylactic acid (PLA) intrascleral implant, the faster *in vivo* versus *in vitro* release rate may have reflected the decrease in pH inside the implant that occurred while it was imbedded in the eye. This drop in pH enhanced the autocatalysis occurring at the center of the polymer matrix. Furthermore, a burst release, which was seen *in vitro*, did not occur *in vivo* [67]. With these examples in mind, the question is whether an *in vitro* test can be devised which adequately models the unique characteristics of the ocular environment.

8. Further questions and conclusion

Despite the wealth of information presented at this workshop, we are still left with many unanswered questions. These include:

1. What variables associated with the formulation and manufacture of in situ forming gels can influence their *in vivo* product release?
2. Can *in vitro* release characteristics predict the *in vivo* performance of long acting lipophilic solutions?
3. When setting expiry for a long acting parenteral product intended to continue releasing for months upon administration, how does one insure that the product will perform in a manner comparable to a fresh product if administered at expiry?
4. Despite the possibility of unique formulations that will require variations in standard test methods, is it feasible to develop standardized *in vitro* test methods that can be applied to the different types of MR parenteral formulations? Or will different mechanisms of release necessitate or allow for the use of different test methods?
5. What unique challenges will be faced when attempting to develop demonstrate *in vivo* bioequivalence of the various categories of parenteral MR products?
6. Is it possible to develop *in vitro* methods for complex targeted delivery systems such as those encountered with ophthalmic MR drug products?

We conclude that these issues should be the subject of continuing dialogue.

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