IN VITRO
RELEASE TESTING

The Issues & Challenges Involved in In Vitro Release Testing for
Semi-Solid Formulations
By: Qiuxi Fan, PhD; Mark Mitchrick, MD; and Andrew Loxley, PhD

INTRODUCTION

The use of an in vitro release test (IVRT) to evaluate drug release from
semi-solid formulations has become the routine test for topical product
development. Like the dissolution test for solid dosage forms, IVRT for
semi-solid dosage has become increasingly important. As FDA
Guidance puts it, “In vitro release is one of several standard methods that
can be used to characterize performance characteristics of a
finished topical dosage form (ie, semi-solids like creams, gels, and
ointments)… A variety of physical and chemical tests commonly
performed on semi-solid products and their components (eg, solubility,
particle size, and crystalline form of the active component, viscosity, and
homogeneity of the product) have historically provided reasonable
evidence of consistent performance. More recently, IVRT has shown
promise as a means to comprehensively ensure consistent
delivery of the active component(s) from semi-solid products. An in vitro
release rate can reflect the combined effect of several physical and chemical
parameters, including solubility and particle size of the active ingredient
and rheological properties of the dosage form. In most cases, in vitro
release rate is a useful test to assess product sameness between pre-change
and post-change products.…”

Important changes in the characteristics of a drug product
formula or the thermodynamic properties of the drug(s) it contains
should show up as a difference in drug release."

Based on FDA Guidance, the
IVRT method for topical dosage
products is built on an open chamber
diffusion cell system like the Franz
diffusion cell system (Figures 1 and
2) with a synthetic polymeric
membrane. The membrane separates
the donor part containing test product
from the receptor part filled with
medium (usually PBS buffer). Diffusion of drug from the topical
product to and across the membrane is
monitored by assay of sequentially
collected samples of the receptor
medium. At predetermined time
points, an aliquot of medium is
removed from the receptor part for
drug content analysis either by high
pressure liquid chromatography
(HPLC) or other analytical technique,
and the same amount of fresh medium
is refilled into the receptor to keep
constant volume. Theoretically, release
is proportional to the square root of
time, ie, a straight line in the release
profile.

This paper discusses different
IVRT set-ups for different systems
(one-phase and two-phase systems) of
topical products and their respective
release profiles, as well as

Figure 1

highlighting the challenges involved
in collecting useful data and how to
overcome them.

ONE-PHASE SYSTEM

There are two one-phase systems
to be discussed: a water-based system,
such as hydroxyethyl cellulose (HEC)
gel with peptide as the API, and oil-
based systems, such as 1-octanol
solution or light mineral oil
suspension of either antibiotic or low
molecular weight agents like lidocaine
or caffeine as the API.

Water-Based System

Two HEC gels with different
concentrations of a peptide API, and a
poloxamer gel with the same peptide,
all containing Transcutol® as a
penetration enhancer have been tested.
using the IVRT method at Particle Sciences Inc. Because of the relative simplicity of the water-based formulations, IVRT was carried out without modification from the FDA Guidance, using the experimental configuration presented in Table 1, and the release profiles obtained from the three formulations are shown in Figure 3.

It is obvious that for water-based one-phase systems, the regular IVRT method works well with no need for modification, and differences between formulation types and API loading within a formulation type are clearly observed.

**Oil-Based System**

Fan et al. investigated the controlled release of an antibiotic drug (clorocycline HCl) from its solution/suspension in an organic solvent through a porous membrane.1 When formulated as a simple system of API solution/suspension in 1-octanol/light mineral oil, IVRT results were also dependent on API concentration in the formulations: 5 mg/ml (Sol. 1) or 10 mg/ml (Sol. 2). A similar IVRT procedure was performed as for the water-based formulations, except that a hydrophilized polyvinylidene fluoride (PVDF) membrane (Millipore, 0.1-micron pore size) was used instead of a nylon one. Table 2 shows the permeation data, and Figure 4 presents the release profiles.1

From these two IVRT examples of different one-phase semi-solid systems, it is not difficult to observe that one-phase systems pose little challenge for the IVRT method mainly because (as the name “one phase” indicates) either a simple diffusion or partitioning is the major mechanism for API transport through the polymeric membrane. Therefore, different formulations are easily distinguished.

**FIGURE 2**

9-Station Franz Cell Stirrer

<table>
<thead>
<tr>
<th>Table 2</th>
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<table>
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<tr>
<th>IVRT Configuration</th>
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<tbody>
<tr>
<td>Diffusion cell</td>
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<tr>
<td>Weight of sample gel</td>
</tr>
<tr>
<td>Membrane</td>
</tr>
<tr>
<td>Receptor medium</td>
</tr>
<tr>
<td>Sampling aliquot</td>
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<tr>
<td>Sampling time</td>
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**TWO-PHASE SYSTEM**

Two-phase systems are more complex than one-phase systems because many more factors are involved, such as API solubility in the two phases, API partitioning between the two phases, interactions within the system and between the emulsion and membrane interface. And these factors might pose challenges for IVRT to differentiate formulations or even to achieve a reliable release profile.

**Oil-in-Water (O/W) System**

The O/W emulsion is the most widely applied system in semi-solid dosage products because of its fast API release, and its relative stability and ease of application to the skin. In most cases, because the API is dissolved in the aqueous continuous phase, there is no major barrier to the API’s transport through the formulation and into and through the polymeric membrane during the IVRT experiment.

At Particle Sciences Inc., several formulations containing the oil propylene glycol (PG), water, and a low molecular weight microbicidal agent such as the API have been tested using regular IVRT conditions. The same IVRT configuration was used except that the receptor medium was a mixture of PBS and ethanol because of this particular API’s low solubility in PBS alone. As shown in Figure 5, formulations of the same concentration of API dissolved/dispersed in different phases were easily distinguished from their IVRT release profiles.

**Water-in-Oil (W/O) System Using Peptide as the API**

In addition to the O/W system, IVRT of water-in-oil emulsions using a higher molecular weight peptide as the API has also been performed at Particle Sciences Inc. Compared to the O/W system, the peptide emulsion system presented the following several challenges for IVRT:
• The high molecular weight of the peptide (close to 2000 Daltons), high solubility in water, and much lower solubility in the continuous oil phase mean that partitioning from the aqueous internal phase into the non-aqueous continuous phase may not be a strong enough driving force for the peptide to diffuse through the membrane.

• The W/O formulation contains a large volume fraction of aqueous phase to dissolve the API, with a relatively small amount of oil-phase components surrounding it as a continuous phase. Within such a tightly bound structure, the peptide may not diffuse from the water phase through the continuous oil phase and release to the medium.

• If negligible release is observed, the IVRT configuration would need to be changed or reformulation with another selection of oil phase and/or emulsifier be carried out.

Initial IVRT was carried out by the routine set-up shown in Table 1. As expected, zero release was observed after 24 hrs, which illustrated the challenges previously outlined. Other research groups also indicated that a solubilized drug's delivery from emulsion systems, such as creams, lotions, or ointments, relies on API's initial concentration, diffusion coefficient in the external oil phase, and partitioning coefficient between the internal water phase and the external oil phase. As for the W/O emulsion system, the preferred partitioning toward the internal water phase would keep the API rarely available in the external oil phase. At the same time, for the API going through the membrane into the aqueous medium, diffusion occurs through the membrane pores filled with medium and is influenced by the partitioning coefficient of the API between the bulk solvent (i.e., the continuous oil phase) and the aqueous solvent in the membrane pores.

In this case, as this high molecular weight peptide API has much higher solubility in water (≥ 100 mg/ml) than in the oil phase (< 10 mg/ml), not surprisingly, partitioning was always favored toward the water phase; therefore, diffusion through the continuous oil phase into the aqueous medium generally was not observed. The major challenge here is that if the continuous phase is different from the aqueous phase containing the API, it would be very difficult for the API to transport through the interface between the carrier fluid and the formulation by diffusion and/or partitioning. In another case, if the API is in a dispersed phase whose continuous phase has a sharp interface with the collection medium, then release will be even lower due to reduction in the diffusion of API through the oil phase, and the fact that the whole formulation will not pass through the membrane.

In order to overcome this delivery challenge, a modified IVRT
configuration was proposed to achieve a measurable release profile from the W/O emulsion system:

- Use a larger pore size (0.8 microns, 1.0 micron) and/or hydrophobic membrane (Celgard membrane, PTFE membrane) to facilitate the emulsion transportation.
- Increase the concentration of API in the emulsion.
- Add organic component to the receptor medium, such as ethanol, to improve wetting the membrane.

After implementing the new set-up, distinguishable release profiles were observed from different W/O emulsion systems.

**SUMMARY**

In the topical pharmaceutical arena, the application of IVRT to investigate drug release rates from emulsion formulations has received increased attention throughout the past decade. This paper analyzed the issues/challenges related to the use of IVRT for different emulsion systems: a one-phase (either oil or water) system and a two-phase (O/W, W/O) system, and whether IVRT can differentiate formulations. One-phase systems and O/W two-phase systems with the API in the aqueous phase (or in the dispersed oil phase but with a non-zero solubility in the aqueous phase) pose little challenge for IVRT with a wide range of membrane choice and medium selection based on API properties. On the other hand, for W/O two-phase systems, the challenges for IVRT are significant and stem from the API solubility issue in the two phases, the API partitioning between the two phases, oil phase membrane-wetting.
issue, and slow release issue. In the case of O/W/O and W/O/W systems, they behave similarly to W/O and O/W systems, with an additional complicating phase.

Differing from case to case, the regular IVRT set-up may need to be modified to meet the requirements of different emulsion systems as well as different APIs. The present paper used a high molecular weight peptide API in a water-in-oil formulation as an example of how to overcome these challenges.

It is evident that the regular IVRT procedure needs to be modified to meet the requirements of different emulsion systems as well as APIs. The present paper used a large MW peptide as an example of how to overcome these challenges based on our successful IVRT experiences for different emulsion systems here at Particle Sciences Inc. Now that IVRT can be adapted to evaluate all types of formulations, the next challenge is the correlation between in vitro and in vivo release results, which is currently under intense investigation at the company.

REFERENCES


BIographies

Dr. Ouard Fan joined Particle Sciences, Inc. in 2006 as a chemist focusing on preformulation and IVRT areas with over 10 years experience in both industrial and academic environments of pharmaceutics and cosmetics. In 2005, Dr. Fan worked at Demik Labs as a Scientist, the dermatology division of Sanofi-Aventis. He published several papers on a variety of topics from passive transdermal/topical delivery to active iontophoretic delivery to applying intelligent polymers (ie, temperature-sensitive gels for transdermal delivery of antibiotics) in the Journal of Controlled Release, Pharmaceutical Research, etc. He is also the inventor of two pending US patents related to transdermal/topical drug delivery. Dr. Fan earned his PhD from New Jersey Institute of Technology.

Dr. Mark Mitchnick is the CEO of Particle Sciences. Dr. Mitchnick holds approximately 20 issued and pending US and international patents related to nanoparticle production, skin care formulations, self-sterilizing catheters, and microcapsulation and stabilization of active ingredients. Dr. Mitchnick earned his BSc in Animal Sciences from Purdue University and his MD from Georgetown University Medical School. He was trained in Pediatrics at The New York Hospital, Cornell Medical Center.

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