Requirements for *in vitro* Performance Testing with a View to *in vivo* Performance

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In vitro – in vivo Relationship

Plasma Concentration (ng/mL)

**Formulation 1**

- **Time (hr)**: 0, 2, 4, 6, 8, 10, 12

- **Percent drug released (%)**: 0, 20, 40, 60, 80, 100

- **Formulation 1**

- **Formulation 2**

**Percent drug released (%)**

- **Formulation 1**

- **Formulation 2**

**in vivo**

**in vitro**
How to correlate?
Experimental test conditions should consider:

- **Characteristics of the dosage form**
  - Route of administration
  - Administered dose
  - Time of administration

- **Chemistry of API**
  - Solubility
  - Stability

- **Physiology**
  - Medium
    - Composition
    - pH value
  - Volume
  - Temperature
  - “Transfer” of dissolved drug
USP’s Taxonomy for Pharmaceutical Dosage Forms

First Tier Categories

Drug Product Classification
(route of administration)

- Gastrointestinal Tract
  - Oral
  - Oropharyngeal
  - Rectal
- Body Tissues or Fluids [by injection]
  - IV
  - IM
  - SC
- Mucosal Membranes
  - Nasal
  - Ophthalmic
  - Otic
- Skin Surface
  - Urethral
  - Vaginal
  - Topical
- Lungs Inhalation
  - Transdermal

## Tier One: Route of Administration

<table>
<thead>
<tr>
<th>Oral</th>
<th>By Injection</th>
<th>Mucosal Membranes</th>
<th>Skin Surfaces</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

## Tier Two: Dosage Form and Physical Properties

<table>
<thead>
<tr>
<th>Solid</th>
<th>Semi Solid</th>
<th>Liquid</th>
<th>Gas</th>
<th>Aerosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>Gels</td>
<td>Solutions</td>
<td></td>
<td>Droplets</td>
</tr>
<tr>
<td>Capsules</td>
<td>Pastes</td>
<td>Suspensions</td>
<td>Gas</td>
<td>Particles</td>
</tr>
<tr>
<td>Powders...</td>
<td>Creams...</td>
<td>Lotions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Tier Three: Type of Release Pattern

- Immediate
- Extended
- Delayed
Physiological consideration

Orally administered dosage forms

- The gastro-intestinal (GI) tract:
  - mouth (oral cavity)
  - pharynx
  - esophagus
  - stomach
  - small intestine
  - large intestine
  - rectum and anus

- Known factors – GI fluids
  - pH-value
  - concentration of salts
  - volume
  - temperature
  - enzymes
  - bile salts

- Unknown factors?
Physiological consideration

Orally administered dosage forms
- The mouth contains
  - teeth and
  - tongue
  - secretions from the salivary glands
- Known factors
  - pH-value
  - concentration of salts
  - Temperature
- Unknown factors?
Physiological consideration

Dosage forms applied on the skin

- The skin is comprised of three layers
  - Epidermis - it is a tough protective layer that contains the melanin-producing melanocytes.
  - Dermis - it contains nerve endings, sweat glands, oil glands, and hair follicles
  - Hypodermis (subcutis) - fatty layer of subcutaneous tissue

- Known factors
  - pH-value
  - Temperature

- Unknown factors?
Simulated Biological Fluids – Oral route

- **Simulated Gastric Fluid – fasted state: FaSSGF**
  - pH 1.6
  - Pepsin: 0.1 mg/mL
  - Na-taurocholate: 80 µM
  - Lecithin: 20 µM

- **Simulated Gastric Fluid – fed state: FeSSGF**
  - pH value: 6 – 3 (early – late)
  - Osmolality: 600 – 300 mOsmol/kg (early – late)

- **Simulated Intestinal Fluid – fasted state: FaSSIF**
  - pH value: 6.5
  - Osmolality: 180 mOsmol/kg
  - Na-taurocholate: 3 mM
  - Lecithin: 0.2 mM

- **Simulated Intestinal Fluid – fed state: FeSSIF**
  - pH value: 6.5 – 5.8 (early – late)
  - Osmolality: 400 mOsml/kg
  - Na-taurocholate: 10 – 4.5 mM
  - Lecithin: 3 -0.5 mM
  - Glyceryl monocholate: 6.5 – 1 mM
Simulated Biological Fluids (1)

- Buccal and sublingual route - Simulated Saliva
  - pH: 6.5 - 7.5

- Parenteral route - Simulated Body Fluid
  - pH 7.2

- Simulated Synovial Fluid
  - pH 7.4

- Pulmonary route – Simulated Lung Fluid
  - pH: 4.5 – 7.4

- Vaginal route – Simulated Vaginal Fluid
  - pH 4.2

- Ophthalmic route – Simulated Tears
  - pH 7.4

(1) Margareth R.C. Marques, Raimar Loebenberg, May Almukainzi Simulated Biological Fluids with possible application in dissolution testing, Dissolution Technologies, 18 (3), Aug. 2011
Unknown factors

- Motility
- Volume
- Change in the composition of biological fluid due to transit
- Transit time
- Absorption through the biological membrane
- Variability from subject to subject
Method Development

- The dissolution procedure requires
  - An apparatus
  - A dissolution medium
  - Operating conditions (rpm, temperature, sampling schedule)

- The method needs to be discriminating yet sufficiently rugged and reproducible for day-to-day operation and capable of being transferred between laboratories
Solubility in various aqueous media

- **pH dependent solubility**
  - Buffer solution with different pH values, e.g. pH 1 to pH 8

- **Influence of salt composition**
  - Different composition of buffer solutions at the same pH value

- **Use of solubilizing agents**
  - To improve wettability
  - To improve solubility

- **Effect of various surfactants (if applicable)**
  - Different surfactant types (e.g. non-ionic, anionic, cationic)
  - Different concentrations of the same surfactant
Biopharmaceutical Classification (BCS)

- **Class I** – high solubility / high permeability
- **Class II** – low solubility / high permeability
- **Class III** – high solubility / low permeability
- **Class IV** – low solubility / low permeability
Selection of Dissolution Medium

- Based on physical and chemical data for
  - Drug substance
    - Solubility of drug substance
    - Sink conditions maintained
    - Stability of drug substance at different pH conditions
  - Drug product
    - Immediate release, delayed release, extended release, transdermal patch, etc.
    - Theoretical drug release mechanism, if known
Dissolution media for in vitro testing

- **0.1 N HCl**
  - Surfactants may be added as needed

- **USP Simulated Gastric Fluid pH 1.2 – SGF**
  - Pepsin may be added as needed
  - Surfactants

- **USP Simulated Intestinal Fluid pH 6.8– SIF**
  - 0.05M phosphate buffer
  - Pancreatin may be added as needed
  - Surfactants may be added as needed

- **Buffer solution pH 4.5**
  - Acetate buffer solution

- **In vitro dose dumping of ER oral drug products**
  - 0.1 N HCl
  - 0%, 5%, 20%, and 40% ethanol added
  - 2 h test with sampling time each 15 min
Selection of Dissolution Medium

- Aqueous buffer solutions pH 1.2 – pH 6.8
  - Modified-release formulations up to pH 7.5

- Reflect the pH conditions at the dissolution site
  Addition of:
  - bile salts (sodium cholate, sodium taurocholate)
  - surfactants (sodium lauryl sulfate, polysorbates)
  - enzymes (pepsin, pancreatin)

- Sink-conditions are desirable

- Purified water as dissolution medium is not recommended
Selection of Dissolution Apparatus

- The choice of apparatus is based on
  - Type of dosage form
  - Formulation design

- For solid oral dosage forms
  - Apparatus 1 and Apparatus 2 are used most frequently

- Agitation can be adjusted as needed
# FIP/AAPS Guidelines for Dissolution/ In vitro Release Testing of Novel/Special Dosage Forms

<table>
<thead>
<tr>
<th>Type of Dosage Form</th>
<th>Release Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid Oral Dosage Forms (conventional)</td>
<td>Basket, Paddle, Reciprocating Cylinder or Flow Through Cell</td>
</tr>
<tr>
<td>Oral Suspensions</td>
<td>Paddle</td>
</tr>
<tr>
<td>Oral disintegrating Tablets</td>
<td>Paddle</td>
</tr>
<tr>
<td>Chewable Tablets</td>
<td>Basket, Paddle or Reciprocating Cylinder with glass beads</td>
</tr>
<tr>
<td>Transdermals – Patches</td>
<td>Paddle Over Disk</td>
</tr>
<tr>
<td>Topicals – Semisolids</td>
<td>Franz Cell Diffusion System</td>
</tr>
<tr>
<td>Suppositories</td>
<td>Paddle, modified Basket or Dual Chamber Flow Through Cell</td>
</tr>
<tr>
<td>Chewing Gum</td>
<td>Special apparatus (Ph.Eur.)</td>
</tr>
<tr>
<td>Powders and Granules</td>
<td>Flow Through Cell (powder/granule sample cell)</td>
</tr>
<tr>
<td>Microparticulate Formulations</td>
<td>Modified Flow Through Cell</td>
</tr>
<tr>
<td>Implants</td>
<td>Modified Flow Through Cell</td>
</tr>
</tbody>
</table>
Selection of the Volume of Dissolution Medium

Basket/ Paddle Apparatus
- generally: 500 ml – 1000 ml
- special cases: 2 or 4 liter
- low dose: 150 ml - 250 ml (not compendial)

Reciprocating Cylinder Apparatus
- Up to 250 ml/vessel
- Use of up to 6 vessels for testing

Flow-through Cell Apparatus
- generally: 4 ml/min – 16 ml/ml
- for implants: 1.0 ml/min – 2 ml/min
- special cases: up to 50 ml/min
Selection of Agitation Rate and Temperature

Stirring rate
- Basket Apparatus: 100 rpm
- Paddle Apparatus: 50 rpm or 75 rpm
- Suspensions: 25 rpm
- Reciprocating Cylinder Apparatus: 5 dips/min – 35 dips/min

Temperature
- Orally administered drugs: 37°C
- Topical/ transdermal dosage forms: 32°C
Oral suspensions for systemic use

- Paddle method, aqueous medium
- Representative sample: reconstituted according to instructions to the patient/practitioner
- Sample weight/volume to reflect typical dose
- Sample introduction and agitation rate based on viscosity and composition of suspension matrix
  - Low viscosity suspensions: Dose delivery to the bottom of the vessel by volumetric pipette; low agitation rate: 25 rpm.
  - High viscosity suspensions: Dose determination by weight with quantitative sample transfer; higher agitation rate: 50 or 75 rpm
- Sample introduction - accurate, precise, and reproducible
- Agitation - discriminate between batches with different release properties
Orally Disintegrating Tablets (ODT) create an in-situ suspension by rapidly disintegrating, typically within one minute or less.

Taste masking is an essential feature of ODT’s and can be a rate determining mechanism for dissolution/release.

Follow the principles of oral dosage forms or suspensions: paddle method at 50 rpm; single point specification.

If taste masking (using polymer coating) is a key aspect of the dosage form, a multi-point profile in a neutral pH medium with early points of analysis (< 5 min) may be recommended.

Only dosage form requiring both disintegration and dissolution testing.
Chewable Tablets

- Generally same procedure as regular tablets
- Same test conditions as for conventional tablets of the same active pharmaceutical ingredient (where applicable)
- Non-disintegrating nature of the dosage form may require different test conditions (e.g., increased agitation rate) and specifications (e.g., increased test duration)
- Reciprocating cylinder with addition of glass beads provide intensive agitation
Suppositories

- Hydrophilic suppositories
  - basket
  - paddle
  - flow-through cell

- Lipophilic suppositories
  - modified basket method
  - paddle with a wired screen and sinker
  - modified flow-through cell with specific dual chamber suppository cell (Ph. Eur. 2.9.3.-6.)

- Temperature: 36°C up to 38.5°C
Liquid-Filled Capsules

Hydrophilic or Lipophilic formulations
- may or may not include a surfactant

- Paddle method with a minimum amount of surfactant, if needed
- Difficulties to keep the formulation immersed
- Modified dual chamber flow-through cell
  - Open system
  - Closed system

- Basket method
- Reciprocating cylinder

- See new USP General Chapter <1094> in Pharm. Forum 38(1) (www.usppf.com)
Transdermal Patches

- Method of Choice:
  - Paddle over disk (USP Apparatus 5) with a watch glass-patch screen sandwich assembly
  - Rotating cylinder (USP Apparatus 6)
  - Reciprocating disk (USP Apparatus 7)
  - Paddle over extraction cell (Ph. Eur. 2.9.4.2)
- pH of the medium ideally pH 5 – pH 6 (physiological skin condition)
- Test temperature: 32°C (skin temperature)
- 100 rpm
- Unnecessary proliferation of dissolution equipment should be avoided
Semisolid Topical Dosage Forms

- Dissolution apparatus
  - Vertical diffusion cell (Franz cell) with synthetic membrane
  - Immersion Cell
  - Modified USP Apparatus 4 cell systems
- With or without using synthetic membranes
- Receptor medium may need to contain alcohol and/or surfactant
- De-aeration critical to avoid bubble formation at membrane interface
- Test temperature: 32°C (skin temperature)
- Sample weight/volume should reflect the typical dose
In vitro Drug Release
Marketed 1% Hydrocortisone Products

- Cortizone 10
- Aveeno
- Hytone
- Fuogera
- Cortaid
- Cortizone 10 Oint.
Injectable Depot Dosage Forms

- Modified flow-through cell
  - low flow rate
  - Tests are often run over a long time period (e.g., several weeks) Take measures against evaporation, microbial contamination
- Osmolarity, pH and buffer capacity of the medium should reflect the site of application (plasma or muscle)
- Challenge:
  - Appropriate duration of the test and sampling times to adequately characterize the release profile
  - Stability of the API over the entire testing time
  - Evaporation of the medium
- Possibility of accelerated test conditions is attractive (elevated temperatures and pH values - faster drug release)
- Verification of validity of the accelerated test conditions
USP App. 4 operated as “closed” system

- 12-mm diameter flow-through cells
- Cells packed with 1 mm glass beads
  (microspheres mixed with glass beads to avoid aggregation)

37.0°C

250 mL of Phosphate buffer saline (PBS) pH 7.4

Filter

- 1.0 µm fiberglass

Flow Rate

- 4 – 35 mL/min
Accelerated in vitro release method by studying effects due to:

- Increased temperature
- Changes in dissolution medium
  - use of hydroalcoholic solutions
  - change in pH value
  - addition of surfactant
- Flow rate
  - varied between 4 and 35 mL/min
- Cell size/type
In vitro Release Profile of a Microsphere Injectable

Real-time drug release profile

USP App. 4 flow rate: 8ml/min, 250 ml phosphate buffer pH 7.4, 37 °C.
USP App. 4 flow rate: 8ml/min, 250 ml phosphate buffer pH 7.4, 45 °C.
Correlation of real time and accelerated release profiles

\[ y = 0.1439x + 0.5091 \]

\[ R^2 = 0.9887 \]
Lack of In Vitro-In Vivo Correlation

- Systemic drug absorption is the rate-limiting step for absorption
  - Variations in drug dissolution/release are not reflected in variations in drug absorption
  - Dissolution is not rate limiting step
- Drug dissolution test is not discriminating
  - May need to modify dissolution conditions
- Other factors affecting systemic drug absorption
  - GI transit time
  - Pre-systemic drug elimination (first pass effects)
  - Enterohepatic circulation
### In Vitro-In Vivo (IVIV) Correlation Expectations for Immediate Release Products Based on Biopharmaceutics Classification System

<table>
<thead>
<tr>
<th>Class</th>
<th>Solubility</th>
<th>Permeability</th>
<th>IVIV Correlation Expectation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High</td>
<td>High</td>
<td>IVIV correlation if dissolution rate is slower than gastric emptying rate. Otherwise, limited or no correlation is expected.</td>
</tr>
<tr>
<td>II</td>
<td>Low</td>
<td>High</td>
<td>IVIV correlation expected if <em>in vitro</em> dissolution rate is similar to <em>in vivo</em> dissolution rate unless dose is very high.</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>Low</td>
<td>Absorption (permeability) is rate determining and limited, or no IVIV correlation with dissolution rate.</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>Low</td>
<td>Limited or no IVIV correlation expected.</td>
</tr>
</tbody>
</table>