**Introduction**

- Aim is to increase drug solubility at physiological pH range and prevent precipitation at injection site
- Some drugs cause Injection Site Reaction (ISR) due to precipitation at the site of injection
- Many drugs (small molecules and biologics) are formulated at pH of highest solubility (away from its isoelectric pH or pI)
- Drugs formulated at a pH significantly away from physiological pH tend to precipitate at the injection site
- Strategy is to formulate drugs to increase solubility at physiological pH to minimize injection site precipitation
- Improving drug solubility at physiological pH enables high concentration doses with lower volume injections that improve patient comfort and compliance

**PBS Dilution Test for Drug Solubility at Physiological pH**

- Prepare formulations for solubility screening with increasing drug concentrations
  - Varying pH and concentrations of solubility enhancing excipients
- Simulate injection by diluting into PBS so final diluted sample reaches physiological pH (>7.0 to <7.5)
  - Evaluate precipitation by visual observation
  - Quantify turbidity over time at 450 nm

**Formulation Dilution in PBS: 450 nm Scatter with and without Excipient 3**

**Example of 450 nm Scatter plots**

- Formulations dialyzed for 4 hours against excess PBS
- Formulation pH change measured
- Turbidity is observed over time at 450 nm

**PBS Dialysis Test for Drug Solubility at Physiological pH- Example Protein Result**

- Formulations dialyzed for 4 hours against excess PBS
- Formulation pH change measured
- Turbidity is observed over time at 450 nm

- Upon dialysis completion, solubility of formulation is evaluated for visual clarity

**Visual Observations:** Increasing color intensity represents increasing turbidity by visual observation or by 450 nm scatter

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INJECTION SITE REACTION
SCREENING METHODOLOGY

Injection Site Reaction Test

Repeated injections:
Seven daily interscapular SC injections of test formulation

Results

Test Article:
1. Formulations with different concentrations and pH
2. Formulations with and without excipients

Biocompatibility:
1. Weight Change (loss or gain)
2. Injection site tissue staining and histology
3. Injection site scoring by pathologist

API activity:
1. Tissue harvest /Activity (~24 hours after last dose)
2. Drug/API Blood Content (~24 hours after last dose)

Injection Site Reaction (ISR) Test:
Biocompatibility and Blood Drug Level

Scoring Tissue Sections for Biocompatibility
- Tissues scored on a scale of 0 to 5 for various lesions:
  i. Necrosis (x3)
  ii. Suppurative (x2.5)
  iii. Mononuclear infiltrate (x2)
  iv. Drug/API precipitate
  v. Fibrosis
- The lesion scores are weighted and summed to score the injection site reaction (ISR):
  i. A score of 0-2 is Normal
  ii. A score of 3-5 is Minimal
  iii. A score of 6-10 is Mild
  iv. A score of 11-20 is Moderate
  v. A score of 21-30 is Marked
  vi. A score >31 is Severe

Histology and Tissue Staining

Normal (Skin: H&E)

Histamine (Mast Cells: Toluidine Blue)

Immunostaining (Skin or target staining)

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