APPLICATION NOTE

Evaluating *In Situ* Forming Gels for Long-Acting Injectables: *In Vitro* Excipient Comparison for Subcutaneous Formulation Development

In situ forming gels enable sustained API release by transitioning from a soluble liquid to a miscible gel upon subcutaneous (SC) injection catalyzing the ability to slowly release a payload over time. Preclinical testing of these formulation types remains challenging due to the limitations of conventional *in vitro* models, where bulk solutions are introduced to sink conditions in a single spontaneous dilution (e.g. USP).

This study utilizes the SCISSOR™ (Subcutaneous Injection Site Simulator) platform to compare five *in situ* f orming gel formulations of the same small-molecule API. Injected into an artificial extracellular matrix mimicking subcutaneous tissue, formulations were analyzed over 7 days using HPLC, imaging, and turbidity data for comparison. Differences in release profiles were highlighted by the SCISSOR assay with Formulation B exhibiting rapid release and limited stability, while Formulation D showed slower release due to precipitation. Images of gelling behavior and subsequent solubilization provided mechanistic insights into each formulation's API release and potential immunogenicity risks.

Within this study SCISSOR effectively differentiated formulation performance, enabling data-driven decisions on gelation, release kinetics, and depot stability to optimize *in situ* forming gel long-acting injectable (LAI) development.

Subcutaneous *In Situ* Forming Gels

In situ forming gels are a commonly used API vehicle for administering LAIs subcutaneously. These formulations begin as liquid solutions containing an active pharmaceutical ingredient (API) and transform into a semi-solid gel upon injection, decreasing solubility of the bulk formulation and enabling extended drug release.[1,2]

Despite their widespread use, evaluating these formulations before clinical studies remains a challenge. It has been reviewed in the literature that animal models (rats, monkeys, minipigs) do not perfectly mimic human physiology, and have been shown to correlate less reliably with clinical outcomes than some *in vitro* methods.[3-5] In addition, standard *in vitro* methods such as USP dissolution, are designed for bulk solution testing and fail to recreate the environment necessary for assessing the soluble-to-insoluble gel formation and subsequent release.

SCISSOR N3 is a fully in vitro platform designed to replicate key physiological properties of human subcutaneous tissue, offering a more predictive and controlled approach for formulation screening, excipient selection, and risk assessment.3-5

In this study, the SCISSOR N3 was used to compare and rank order different in situ forming gel formulations of the same API, providing valuable insights for optimizing the formulation constituents during in situ forming gel drug development.

Pion

Methods

Five blinded *in situ* forming gel formulations (Samples A–E) containing the same small molecule API with varying excipients were analyzed using the SCISSOR system (Pion Inc.) (Fig. 1). SCISSOR features a cartridge filled with an artificial extracellular matrix (ECM) that mimics an acellular subcutaneous environment. 3-5 Each formulation was injected into the artificial ECM using a standard hypodermic needle (25G), where it began its gelation process, equilibrated after curing, and eventually released API through customized dialysis membranes. The released API then entered an outer chamber containing a biorelevant bicarbonate buffer with 0.02% sodium azide, simulating systemic circulation uptake. The assay was conducted over 7 days to compare release profiles across formulations.

API concentrations in the receiving chamber were quantified via offline HPLC analysis (Acuity Arc, Waters), with calibration curves generated from serial dilutions of a 1 mg/mL API solution in methanol (0–327.33 µg/mL). These concentrations were converted to %Release or comparative ranking. Additionally, SCISSOR's turbidity sensors and cameras monitored injectate behavior within the ECM cartridge throughout the experiments.

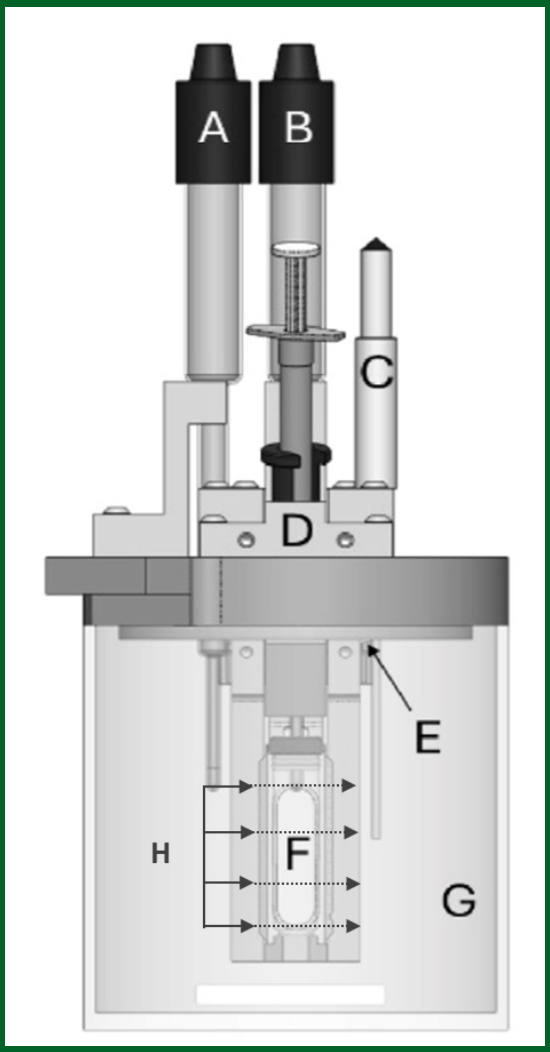

Figure 1. Labeled schematic of SCISSOR chamber: A) Chamber pH meter, B) injection site pH meter, C) Thermocouple, D) Injection port, E) CO2 (g) port, F) ECM cartridge, G) Chamber , H) Turbidity sensors.

Results and Discussion

Injection Behavior

Five *in situ* forming gel formulations containing the same small-molecule API were evaluated using the SCISSOR platform over 7 days. These LAI formulations were expected to swell upon injection and hydration, but their actual behavior varied. (Fig 2).

Formulation B demonstrated the most representative swelling behavior with the turbid injection appearing throughout the majority of the cartridge post-injection. Formulations A and E showed phase separation with the injectate appearing to concentrate on the lower half of the cartridge and miscibility was seen in the upper half, followed by incomplete release of the turbid material.

Formulation C initially migrated throughout the artificial ECM before precipitating at the bottom of the cartridge. Whereas formulation D immediately formed a precipitate at the injection site, but achieved miscibility before 1 day post-injection.

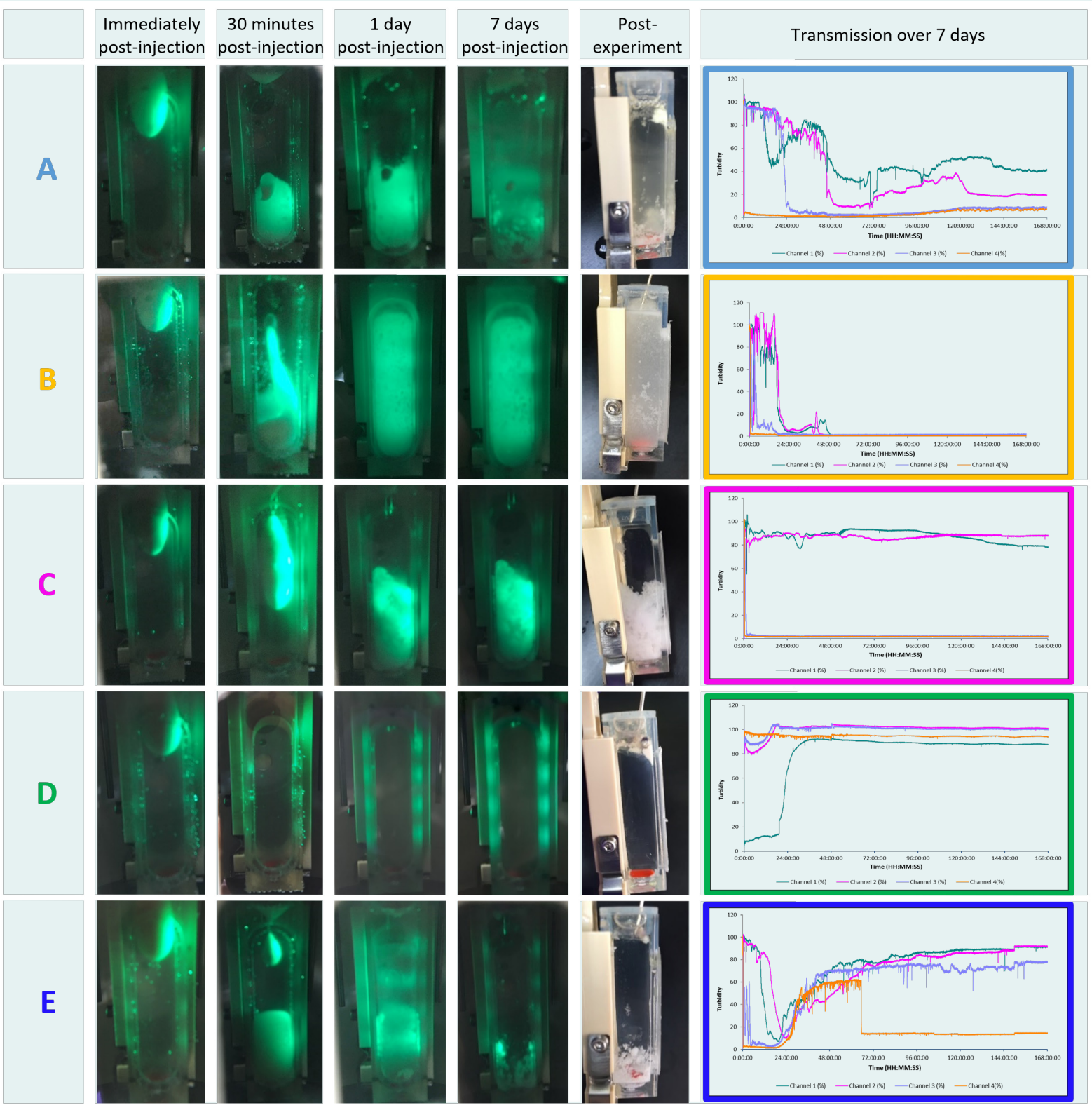

Figure 2. Photographic data showing formulations A, B, C, D and E immediately-, 30 minutes-, 24 hours- and 7 days-post injection, and after the experiment was complete as well as the individual turbidity graphs for each formulation.

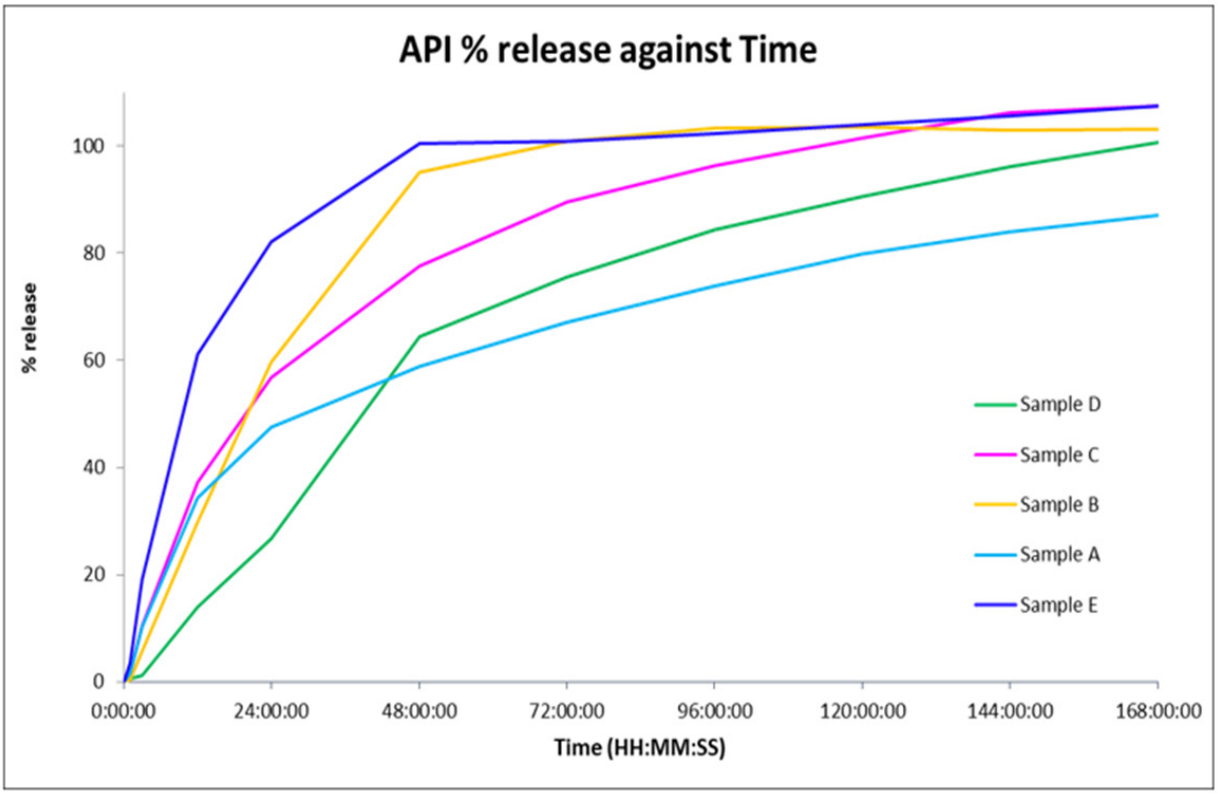

Figure 3. % Release data over 7 days for all five formulations.

Release Comparison

Formulation A exhibited the slowest release, likely due to precipitation at the injection site and material persisting within the ECM cartridge through the duration of the experiment.

Formulation B, despite swelling throughout the cartridge, API released rapidly and reached peak levels within 48 hours, suggesting that the excipients of this formulation allow for complete gelling and swelling of the solid architecture while still allowing for uninterrupted diffusion of the API.

Formulation C showed a gradual release, influenced by precipitate dissolution visible in the lower half of the cartridge. Formulation C never achieved full miscibility, likely explaining its deviation from Formulation E's release profile during the first 48 hours.

Formulation D showed the longest lag phase and slowest initial release post-injection. Based on the images shown in figure 2, the persistence of the bolus in the top portions of the cartridge suggest that not only was there less API being released due to solubility barriers, but the passive diffusion and swelling of any material seems to be inhibited by the specific excipients within this injectate.

Formulation E exhibited the sharpest release rate, despite the presence of turbid material in the ECM cartridge - typically a sign of gelation and slowed release. In this case, the turbid phase remained solution-like, with no distinct aqueous–gel boundary forming. If a solid gel failed to form, this would likely allow for rapid diffusion of the payload from the ECM cartridge.

Conclusions

SCISSOR effectively differentiates *in vitro* performance of *in situ* forming gels, providing insights into how excipient variations impact gelation and API release. While release data alone can help identify sustained-release candidates, imaging data enhances mechanistic understanding by revealing factors like precipitation and boundary layers that could increase immunogenicity risks and formulation development decisions. This data highlights how imperative it is to couple release data with behavioral data to understand formulation performance and risk.

References

1. Omidian, H., et al. Long-Acting Gel Formulations: Advancing Drug Delivery across Diverse Therapeutic Areas, Pharmaceuticals, 17 (4), 493 (2024).
2. Pandya, A. K., et al. *in Situ* Forming Depots for Long-Acting Drug Delivery Systems. Advanced Drug Delivery Reviews, 200, 115003 (2023).
3. Bown, H. K., et al. *In Vitro* Model for Predicting Bioavailability of Subcutaneously Injected Monoclonal Antibodies. Journal of Controlled Release, 273, 13–20 (2018).
4. Gomes, C., et al. Hydrogel Formats to Model Potential Drug Interactions Occurring at the Subcutaneous Injection Site. European Journal of Pharmaceutics and Biopharmaceutics, 199, 114308 (2024).
5. Hanafy, B. I. et al. Predicting human subcutaneous bioavailability of monoclonal antibodies using an integrated *in vitro/in silic*o approach. Journal of Controlled Release, 380, 715-724

Pion
+1 978.528.2020 | +44 (0) 1342 820720
sales@pion-inc.com
www.pion-inc.com