U.S. FOOD & DRUG ADMINISTRATION

Biosimilar User Fee Act (BsUFA) III Regulatory Science Pilot Program

ANNUAL REPORT

Contents

Check if this report is Progress or Final Report:

☒ **Progress report** ☐ **Final report**

1. Report Overview

The foundation of a biosimilar manufacturer's regulatory filing is the demonstration of an analytical and functional similarity between the biosimilar product and the appropriate originator product. The interference that the excipients in the formulation cause during the standard range of analytical and functional techniques is one of the most difficult challenges that must be overcome while performing these operations. As a consequence, producers of biosimilar products resort to a variety of approaches to isolate the biotherapeutic protein from the drug product formulation. However, there is an element of uncertainty regarding the impact that this isolation has on the stability of the active pharmaceutical ingredient (API) and the findings that are produced afterward. In light of these obstacles, the purpose of this project is to develop an analytical platform that will enable us to carry out trustworthy characterization and evaluation of the comparability of biosimilar products in lyophilized and liquid formulations. The potential interference the excipients cause during analytical and functional characterization is being systematically investigated. We are also identifying the challenges and potential incompatibilities in the employment of analytical methods. We are assessing the similarity of products made of a single API but formulated as a variety of commercially available biosimilars. Our goal is to establish the effect of different excipients on the critical attributes of a single API. We are therefore (i) comparing the stability of a monoclonal antibody in different formulations against various physical stresses (freeze-drying, freeze-thaw, agitation) and (ii) the effect of formulation composition on the storage stability of the API. We have established the effects of higher trehalose concentration, a non-reducing sugar commonly used as a stabilizing agent in biotherapeutic formulations, on formulated products under different stress conditions. Trastuzumab (Tmab) is the model protein for our studies.

Table 1: High-level overview of the project objective, aim(s) progress, outcomes, and timelines for communication and regulatory impact (1-2 sentence max per table cell).

Project Title:	Platform for reliable characterization and evaluation of comparability of biosimilar drug products in lyophilized and liquid formulations
Investigators:	1. Raj Suryanarayanan; surya001@umn.edu 2. Vadim Gurvich; vadimg@umn.edu 3. Reza Nejadnik, reza-nejadnik@uiowa.edu 4. Anurag Rathore; asrathore@biotechcmz.com
Organizations:	National Institute for Pharmaceutical Technology and Education (NIPTE)
Grant No. (if applicable)	1U01FD007758-01
Project Objective:	To develop a platform for reliable characterization and evaluation of comparability of biosimilar drug products in lyophilized and liquid formulations

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Assess the similarity of products made of a single active pharmaceutical ingredient (API) but formulated as a variety of commercially available biosimilars	The work has been completed and we are in the process of interpreting the results and writing the manuscripts. The tentative titles of the manuscripts are: (i) Comparative stability of a monoclonal antibody in different formulations against various physical stresses (freeze-drying, freeze-thaw, agitation) (ii) Effect of formulation composition on accelerated stability of Tmabs	The formulation composition influenced the stability of trastuzumab (Tmab) against freeze-thaw and agitation stress in Herceptin® composition but not in the Herzuma®, Ogivri® and Tranzimera® compositions.	Submit the manuscript for FDA review by August 1, 2024

Specific Aim(s)	Progress	Outcomes	Communication Timeline
2. Identify the challenges and potential incompatibilities in the employment of the analytical methods which are typically used for testing the similarity and compar-ing the biosimilars with an innovator product.	The work is complete. Data analysis is almost complete. Paper writing in progress. Tentative title: “Removal of excipients interferes with certain analytical and functional methods used for product characterization and for comparability studies”	Interference of certain excipients is seen with some methods (FTIR, CD, fluorescence and CEX) while interference is not observed for other methods (SEC, SPR)	Submit the manuscript for FDA review by end of July 2024
3. Establish the effect of different excipients and container closure components on the critical attributes of a single product.	The work is complete and we are in the process of interpreting the results and writing the manuscript. The tentative title of the manuscript is: “Effect of formulation composition on accelerated stability of Tmab.”	In the accelerated stability studies (50 °C; 2 months), Tmab in the Herceptin composition showed lower stability than the other compositions.	Submit the manuscript for FDA review by September 15, 2024
	We are working on a review/white paper that investigates the use of different excipients and container-closures for biosimilars in the US market and address the guidelines versus the reasons behind different choices by biosimilar manufacturers.	Choice of excipients and container closure varies from company to company suggesting multiple factors other than the stability are considered.	Submit the manuscript for FDA review by October 31, 2024

2. Progress Summary

The three NIPTE member institutions - The University of Minnesota, The University of Iowa and The Indian Institute of Technology (Delhi) work closely and collaboratively. However, every institution takes the lead on one or more projects.

Please note: The appendix and its references were redacted at the request of the awardee.

Summary of work carried out at IIT Delhi

Project Objective: The proposed project consists of three specific aims and studies are in progress in the following areas:

- To establish the impact of excipient extraction on mAb stability during buffer exchange and excipient extraction process (completed and published). An excipient extraction study was conducted with the objective of establishing the impact of excipient extraction on mAb stability during the buffer exchange process (Published in Molecular Pharmaceutics in March 2024).
- To establish the interference of excipients during analytical and functional characterization of biotherapeutics (on-going).
- To establish the effects of high trehalose concentration on mAb products under different stress conditions. The object is to offer insights into the stability effects of high trehalose concentration (110 mM) on liquid trastuzumab (Tmab) under forced stress conditions including thermal, light, accelerated

stability and extraction stresses. This study has been concluded and the manuscript communicated for publication.

Summary of work carried out at the University of Minnesota

- To establish the stability of a monoclonal antibody in different formulations against various physical stresses (freeze-drying, freeze-thaw, agitation) (ongoing).
- To determine the effect of formulation composition on the storage stability of trastuzumab (Tmab) (ongoing).

Summary of work carried out at the University of Iowa

- To establish the challenges associated with the effective surfactant removal from antibody formulations.
- To write a comprehensive review article on biosimilar formulation.

3. Research Outcomes

Research outcomes of studies performed at IIT Delhi

Study 1. Impact of excipient extraction and buffer exchange on recombinant monoclonal antibody stability

The foundation of a biosimilar manufacturer's regulatory filing is the demonstration of analytical and functional similarity between the biosimilar product and the pertinent originator product. The excipients in the formulation may interfere with characterization using the typical analytical and functional techniques during this biosimilarity exercise. Consequently, the producers of biosimilar products resort to buffer exchange to isolate the biotherapeutic protein from the drug product formulation. However, the impact that this isolation has on product stability is not completely known. This study aims to elucidate the extent to which mAb isolation via ultrafiltration-diafiltration based buffer exchange impacts mAb stability. It has been demonstrated that repeated extraction cycles do result in significant changes in higher-order structure (redshift of 5.0 nm in fluorescence maxima of buffer exchanged samples) of the mAb and also an increase in formation of basic variants from 19.1% to 26.7% and from 32.3% to 36.9% in Extracted Innovator and Biosimilar Tmab samples, respectively. It was also observed that under certain conditions of tertiary structure disruptions, Tmab could be re-stabilized depending on formulation composition. Thus, mAb isolation through extraction with buffer exchange impacts product stability. Based on the observations reported in this paper, we recommend that biosimilar manufacturers take into consideration of these effects of excipients on protein stability when performing biosimilarity assessments.

Study 2: High concentration of trehalose dihydrate stabilizes trastuzumab under forced stress conditions

Stability of complex biotherapeutics like monoclonal antibodies is paramount for their safe and efficacious use. Excipients are inactive ingredients that are added to the purified product so as to offer it a stable environment. Trehalose dihydrate is a non-reducing sugar that is commonly used as a stabilizing agent in biotherapeutic formulations under liquid and frozen states. The stabilizing effect of trehalose against aggregation in lyophilized protein formulations is well known. The present study aims to offer insights into the stability effects of high trehalose concentration (110 mM) on liquid trastuzumab (Tmab) under forced stress conditions including thermal, light, accelerated stability and extraction stresses. Under thermal stress, while high molecular weight (HMW) accounted for 4.82% in the Tmab sample without trehalose, it was 2.39% at high trehalose concentration. Similarly, under light stress, the Tmab sample without trehalose had 35% more HMW than at high trehalose concentration. Likewise, accelerated and extraction stress induced secondary and tertiary

structure disruptions were reduced at higher trehalose concentration. Overall, Tmab stability under forced stress conditions improved significantly at higher trehalose concentrations.

Study 3: Excipient removal interferes with analytical and functional methods used for product characterization and for comparability studies

An extensive analytical and functional characterization of a biotherapeutic product is a regulatory requirement, more so for biosimilar products where approval is contingent on the manufacturer's ability to demonstrate comparability of their product to the corresponding reference product. However, typical biotherapeutic formulations contain multiple excipients, meant to stabilize the product. It is known that some of these excipients can interfere with certain analytical and functional techniques that are typically used in the above-mentioned characterization and comparability exercises. This interference can influence the outcomes of the product stability and comparability studies that are routinely performed as part of product development. In this study, we aim to understand the interference caused by the various excipients that are present in recombinant trastuzumab reference product and its commercially available biosimilars. To examine this interference, excipients were removed one at a time from the drug product and impact of this removal on a spectrum of analytical and functional tools was examined. Removal of certain excipients was found to impact the outcome of analysis by CE-HPLC (for charge variant analysis), far-UV CD, near-UV CD, FTIR, and intrinsic fluorescence analysis. In view of these results, it is recommended that biosimilar manufacturers must ensure parity of concentrations in the samples that are being compared as well as the impact of excipient removal if they are extracting the therapeutic moiety from the drug product for comparability analysis (routinely done by biosimilar manufacturers). Awareness of these concerns will likely contribute to the development of safe and efficacious biotherapeutic products.

Research outcomes of studies performed at the University of Minnesota

Study 1: Comparative stability of a monoclonal antibody in different formulations against various physical stresses (freeze-drying, freeze-thaw, agitation)

Monoclonal antibody formulations are designed to provide conformational and colloidal stability to the API throughout its shelf-life. Biosimilar developers have used different formulation compositions for the same API and the excipient selection is most likely based on the API intrinsic properties like glycosylation level, isoelectric points etc. However, the impact of formulation composition on the stability of a given API against different stress conditions is still not comprehensively understood. We have evaluated the effect of different formulations on structural and colloidal stability of a model monoclonal antibody against three most common stresses - freeze-thaw, freeze-drying, and agitation. These stresses are encountered during the production, storage (both of the drug substance and the final drug product), and formulation of the API into a lyophilized product -. Our results indicate that formulations with a high trehalose content showed good conformational as well as colloidal stability against the different stress conditions. However, when the trehalose concentration was reduced, the formulation exhibited reduced conformational stability during freeze-thaw and agitation stress. Sucrose based formulation) provides good conformational stability against all three stresses. Freeze-drying caused a significant increase in soluble aggregates. Sorbitol based formulations remained structurally stable under all three stress conditions. However, its colloidal stability got compromised under agitation stress wherein significant increase in subvisible particles was observed. Our findings suggest that biosimilar developers, during formulation design, should consider both the conformational and colloidal stability of monoclonal antibody.

Study 2: Effect of formulation composition on the storage stability of trastuzumab

Therapeutic monoclonal antibody (mAb) products must be stable over the long term in order to be successfully commercialized. It is important to understand the possible degradation pathways that may hamper the drug's safety and efficacy upon long term use. The impact of extended storage on the product should be assessed as

a function of temperature since variations in drug thermal stability over time have been linked to the risk of high molecular weight species formation, which may evoke immune response. In general, long-term stability of protein in a given environmental condition is the direct function of its physicochemical properties and solvent compositions. This particular study has focused on investigating the effects of formulation compositions on long term stability of a model monoclonal antibody, trastuzumab (Tmab). Herein, the API was extracted from a commercial product through buffer exchange method and reformulated into four different formulations (F1, F2, F3 and F4). The formulations were stored at two temperatures, 40 and 50°C for the period of one and two months. The formulations were characterized using an array of analytical methods including far-UV CD, near-UV CD spectroscopy, fluorescence spectroscopy, dynamic light scattering (DLS), size-exclusion chromatography (SEC) and flow imaging microscopy. In all formulation compositions, following storage at 40°C after two months, Tmab showed reasonably good tertiary structure stability. However, partial secondary structure destabilization was observed for Tmab in formulation composition F1. Upon storage at 50°C for one month, both tertiary and secondary structural destabilization of Tmab was observed in all formulation compositions. Surprisingly, Tmab retained good conformational and colloidal stability in all formulations after storage at 50°C for two months. Further investigations are in progress to understand this anomalous behavior.

Research outcome of studies performed at the University of Iowa

Study 1: Challenges with the effective removal of surfactants from Tmab formulations

Buffer exchange is a critical step in biologics development, playing a pivotal role in removing contaminants, adjusting sample conditions, and facilitating compatibility studies. This study explores the efficiency of centrifugal concentrators in polysorbate removal compared to a two-step approach involving a surfactant removal column followed by buffer exchange. Trastuzumab-pkrb from Herzuma® served as the model protein. Results demonstrate that a 30 kDa centrifugal concentrator is insufficient for polysorbate removal, whereas a 50 kDa concentrator exhibits partial removal. Surfactant removal column proves most effective but leads to pronounced protein loss. The study investigates the impact on stability, revealing no significant aggregation or conformational changes. Buffer exchange using polysorbate-containing formulation buffer, even with a 50 kDa concentrator, accumulates polysorbate, emphasizing the need for a different approach in small-scale formulation. Adding polysorbate in a separate step after buffer exchange appears to be a good strategy to prevent this problem. The comparison between the two formulation approaches does not show significant aggregation. However, the stability of Tmab secondary structure seems to be affected especially in formulation with high polysorbate content.

Study 2 (review article): Review on biosimilar formulations.

We will provide the development patterns and historical background of biosimilar compositions. The several types of formulations (lyophilized, solutions, etc.) will be summarized. We will comprehensively discuss the excipients used in biosimilar formulations with an emphasis on the frequently used excipients which function as stabilizers (sucrose, trehalose), surfactants (polysorbates), buffers (histidine, phosphate), and bulking agents (mannitol, glycine).

4. Regulatory Impacts

Establishing analytical comparability is an obligatory step for filing for regulatory approval, involving both analytical and functional characterization of the biosimilar. The interference caused by the presence of excipients in the formulation is a major hurdle when evaluating the comparability of the drug products with biosimilar manufacturers resorting to a variety of methods for isolation of the biotherapeutic protein from the drug product formulation. This is why in this study we examined the impact of the destabilization caused by excipient addition/removal to the product. In this study we have focused on the impact with respect to charge variant profiles and non-reversible degradation of secondary and tertiary structures in innovator and biosimilar products. We demonstrate that repeated extraction does have a significant impact on product quality and is likely to impact product stability as well. Hence, we recommend that perhaps the best practice would be for

biosimilar manufacturers to first make the drug product for their biosimilar and then extract the protein from the drug product like they do for innovator product for an equitable comparison.

In order to receive regulatory approval for biosimilar products, manufacturers need to demonstrate that their products are analytically and clinically comparable to the original product. This is an obligatory step that needs to be taken in order to offer assurance about the quality, efficacy, and safety of the product under consideration. During this procedure, both the structure and the function of the biosimilar are characterized. The interference that the excipients in the formulation exhibit with the typical analytical and functional techniques that are employed for characterization and for comparability assessment of biosimilars, is a significant challenge that needs to be overcome. Mischaracterization due to such interferences could lead to incorrect conclusions about the protein's stability as well as its comparability to the reference product. Therefore, it is crucial to ensure that the analytical methods are capable of distinguishing between structural changes and artifacts caused by sample preparation or excipient variations.

Formulation for biotherapeutic products is a topic that is subject of a lot of patents. As a result, biosimilar producers often struggle to identify a stable formulation for their product that does not conflict with an existing patent. We demonstrate how a higher concentration of trehalose (100 mM) offers significantly greater stability in the case of trastuzumab. Since trehalose is an excipient that has been already used in approved commercial formulations, it could potentially be used as an excipient more widely for mAb formulations.

Conventionally, in biotherapeutic formulations, the API used by the different manufacturers are not “identical”. Therefore comparison of products from different manufacturers becomes challenging. In order to overcome this problem, we used the Tmab from one source and prepared formulations simulating the compositions of the innovator product and several biosimilars. We have thus delineated the impact of different formulation compositions on the structural and colloidal stability of API subjected to three different stresses - freeze-drying, freeze-thaw, and agitation. Interestingly, the biosimilar formulation compositions, in general, appear to be very effective in withstanding the stresses during product manufacture and use.

5. Communication and Dissemination

Published and submitted

Sarin, D.; Krishna, K.; Nejadnik, M. R.; Suryanarayanan, R.; Rathore, A. S. Impact of Excipient Extraction and Buffer Exchange on Recombinant Monoclonal Antibody Stability. Mol. Pharm. 2024, 21 (4), 1872–1883. https://doi.org/10.1021/acs.molpharmaceut.3c01157.

Sarin, D.; Krishna, K.; Chakraborty, D.; Sreenivasan, S.; Rathore, Higher concentration of trehalose dihydrate stabilizes trastuzumab under forced stress conditions, 2024, J. Pharm Sci., paper submitted and under review

Presentations

AAPS PharmSci360 conference, Orlando, FL 2023. Poster presented: Investigating Stability of Trastuzumab (Herzuma®) Undergoing Lyophilization and Freeze-Thaw Stresses by Refaat H, Ahmad A, Rathore AS, Suryanarayanan R, Nejadnik R

2024 IPRIME Annual Meeting, 2024 University of Minnesota: Oral presentation: Effect of formulation composition on the stability of trastuzumab against physical stresses by Aziz A, Refaat H, Nejadnik R, Rathore AS, Suryanarayanan R

Under preparation

The tentative titles of the manuscripts are listed below along with the corresponding author in parenthesis.

1. Excipient removal interferes with analytical and functional methods used for product characterization and for comparability studies (A. Rathore).

2. Stabilizing effects of trehalose (A. Rathore).
3. Comparative stability of a monoclonal antibody in different formulations against various physical stresses - freeze-drying, freeze-thaw, agitation (Raj Suryanarayanan).
4. Effect of formulation composition on the storage stability of (Raj Suryanarayanan).
5. Challenges with the effective removal of surfactants from Tmab formulations. This manuscript is complete (Reza Nejadnik).
6. Review on biosimilar formulations (Reza Nejadnik).
7. It is also quite likely that we will have a few more manuscripts based on the work to be conducted until the end of the funding.

6. Challenges

With relation to the interference study, the basic challenge was to establish interference in spectroscopic tools arising specifically from excipients and not due to destabilization of the mAb itself. For this, separate spectral plots (Figure 4, 5 and 7) of buffer blanks, buffer blanks with mAb and mAb (subtracted) spectra were plotted for three type of samples: (a) no excipient sample (milliQ (MQ)); (b) all excipient sample (DP) and (c) only one excipient. The buffer plots depicted excipient footprints in the spectra at specific wavelengths in the spectra which were also reflected in other spectra plots with excipient combinations, but not for the spectral plots of sample without any excipient. If the variations were due to destabilization of the mAb, differences in spectra would have followed unique trends at varied wavelengths/wavenumbers for each sample rather than the variations specific to excipient footprints.

Regarding Aim 3, we included the topic of container closure in the review of the products in the market but did not study the effects of different container closures experimentally. This decision was made in consultation with the FDA team in order to focus the efforts and resources on the other sections and make them more relevant to the biosimilar developers. The research team recognized the significance of the contribution to regulatory aspects and biosimilar development and was happy to steer the focus from scientific finding-oriented research to more regulatory science and development-related areas which was obtained after several rounds of discussion with the FDA scientific panel.

7. Next Steps

1. Future Plans (IIT Delhi)

- To complete and compile the results of the interference study into a manuscript and submit for publication.
- Public communication of results of the current research through international conferences.
- Initiate the experimental plan on alternate analytical characterization tools for cases where excipient interference is being observed.

2. Future Plans (University of Minnesota)

- Impact of aggressive freeze-drying on the stability of trastuzumab in different formulation compositions.
- Accelerated stability of reconstituted trastuzumab lyophiles.
- Communication of research outcomes in international journals and conferences.

3. Future Plans (University of Iowa)

- Comparison of DLS and NTA for characterization of biosimilars at the DS and DP level.
- Publication of the review and the research papers in international journals

8. References

1. References used in IIT Delhi work

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2. References used in University of Minnesota work

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3. References used in University of Iowa work

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