# Chapter 9
# Stability studies

# 9 Stability studies

## 9.1 Introduction

Stability studies are aimed to provide idea about the change in performance and quality of drug products with time when exposed to environmental factors like humidity, temperature and light. This also helps to establish shelf life of product and storage conditions, which according to FDA, is to be indicated on the label. Currently, stability studies are considered pre-requisite for regulatory filing and approval [1]. ICH and WHO stability guidelines have established harmonization in application of stability study and thus it is widely acceptable worldwide [2, 3].

The advent of stability study is done by performing forced degradation studies [1] which helps to identify degradation products and also assist in analytical method development. Later, accelerated stability testing is performed to estimate excipient compatibility in the formulation, shelf life and storage condition of the developed formulation which are required parameters for toxicological and clinical studies. Finally, stability study also comprises of long term study which estimates real time stability of the formulated product. According to ICH guidelines, first three commercial batches are also placed for stability studies. Additionally, if there is change in process, excipient etc. repeat stability study needs to be performed.

Recently, concept of science and risk based approach for stability by application of QbD (Quality by design) principles have facilitated user to limit some unnecessary stability studies. This deals with application of scientific principles to understand and control factors that affect stability performance of the product. Alternatively, several mathematical models are being developed which can accurately predict stability of the proposed formulation [4]. Application of such innovative techniques allows scientifically robust and sound stability space determination which could also predict changes necessary in process, scale and other parameters. This may also lead to continuous improvement in quality of product without waiting for long term stability data. Detailed guidelines and requirements for regulatory filing can be referred from ICH Q1A(R2) guidelines which provides comprehensive guide on performing stability studies. This stability study protocol was decided as per ICH Q1A(R2) guidelines for zone III and zone IV countries [5, 6], still the time period was taken as 3 months to get idea about stability of the prepared formulations. The conditions for storage of prepared formulations were decided as per ICH guidelines which were as follows:

Table 9. 1: Storage conditions of prepared formulations for stability study

| Study | Storage condition |
|---|---|
| Accelerated study | Temperature - 25 ± 2 $^0$C<br>Relative humidity - 60 ± 5% RH |
| Long term study | 5 ± 3 $^0$C |

## 9.2 Procedure

### 9.2.1 Stability study of formulated polyplexes and lipopolyplexes

Stability study of prepared polyplex and lipopolyplex was done at following conditions as per ICH guidelines – accelerated study (25 ± 2 °C/ 60 ± 5% RH) and long term study (5 ± 3 °C) for 3 months. For study, weighed quantity of the prepared lyophilized polyplex and lipopolyplex formulations was transferred to type I glass vials with chlorobutyl rubber stopper and finally sealed with aluminum seals. These were stored at above mentioned storage conditions and sampling was done at predefined sampling intervals. The sampled formulations were analyzed for siRNA integrity and particle size distribution.

### 9.2.2 Stability study of nasal spray

10 gm of nasal spray was filled in the CPS Technology Platform spray pump (Aptar Pharma, Illinois, USA) and stored at 5 ± 3 °C for 3 months. Sampling was done at predefined sampling intervals and the samples were analyzed for various parameters including pH, osmolality, viscosity, pump delivery volume, plume geometry and droplet size distribution.

## 9.3 Result and discussion

### 9.3.1 Stability of polyplexes and lipopolyplexes

Figure 9. 1 demonstrates the results of stability study by determining % recovery and particle size of polyplexes and lipopolyplex, stored at 2-8 °C. The data concluded that there was no significant change in both % recovery and particle size of both the formulation proving siRNA integrity in the conjugated form and physical stability of polyplex and lipopolyplex in terms of particle size. Furthermore, particle size data also proves stability of formulation against aggregation. Conversely, % recovery of siRNA from formulations stored at accelerated condition was drastically reduced to ~70% after 1 month and thus the stability study was discontinued at that condition.

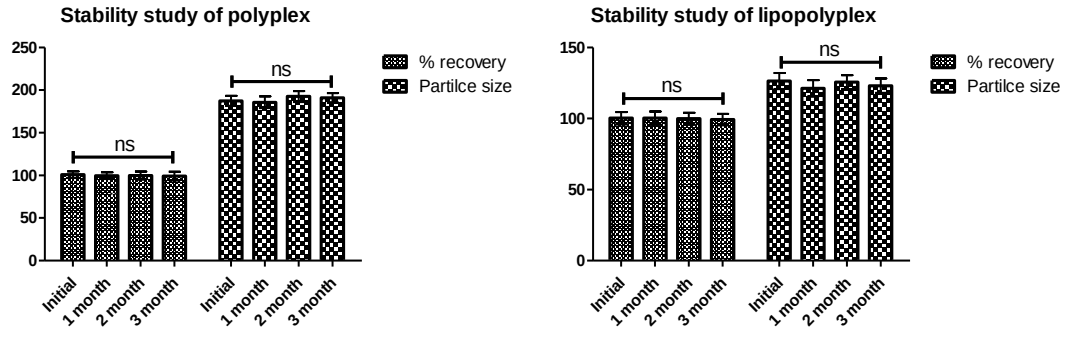


Figure 9. 1: % recovery and particle size of polyplex and lipopolyplex at various time points

### 9.3.2 Stability of nasal spray

The results of physicochemical parameters of nasal spray included in stability study are depicted in Table 9. 2.

Table 9. 2: Stability data of physicochemical properties of nasal spray

| **Parameter** | **Time point (months)** | | | |
|---|---|---|---|---|
| | **Initial** | **1 month** | **2 months** | **3 months** |
| pH | 5.12 ± 0.01 | 5.26 ± 0.04 | 5.14 ± 0.03 | 5.09 ± 0.01 |
| Osmolality (mOsmol/kg) | 535 ± 5 | 526 ± 2 | 538 ± 7 | 533 ± 7 |
| Viscosity (cP) (10 RPM) | 40.5 ± 1.5 | 40.1 ± 1.1 | 39.6 ± 0.8 | 40.8 ± 2.2 |
| Pump delivery volume (µl) | 73.52 ± 0.99 | 74.71 ± 0.57 | 73.88 ± 0.88 | 74.22 ± 0.96 |

The data of physicochemical characterization indicated stability of formulated nasal spray in terms of pH and other physicochemical parameters. pH and osmolality have important role in maintaining nasal homeostasis simultaneously affecting nasal permeability. Similarly, viscosity and pump delivery volume have important role in regulating dosing uniformity. Thus, maintenance of all such parameters proves important and drastic changes in such parameters may sometimes render the therapy ineffective. Additionally, plume geometry of nasal spray was determined after 3 months, as mentioned previously, and the results are demonstrated in Figure 9. 2. Initial results are not shown here as they are already represented in Figure 7. 5. The data showed that plume angle and plume width were 32.6 ± 3.8° and 20.3 ± 1.1 mm, respectively at 3 cm. Similarly, plume angle and plume width at 6 cm were 27.6 ± 0.7°

and 29.5 ± 0.3 mm, respectively. The data of plume geometry represented that there was no significant change in plume geometry of nasal spray even after 3 months indicating its stable spray characteristics in turn providing reproducible nasal spray deposition and therapeutic effect.

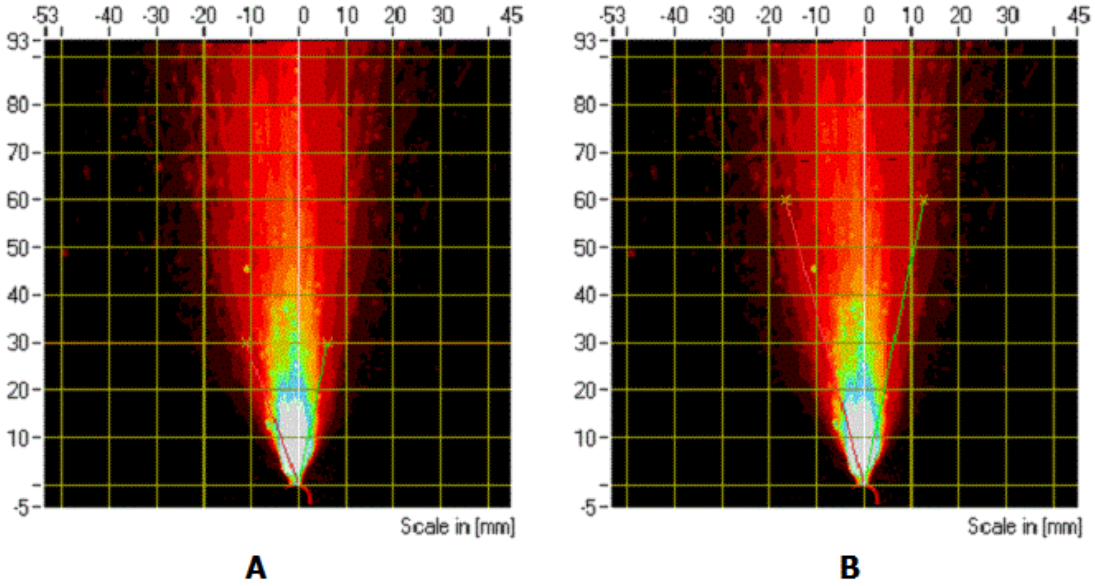


Figure 9. 2: Plume geometry of nasal spray after 3 months at (A) 3 cm and (B) 6 cm

Furthermore, droplet size distribution results at predetermined time points is demonstrated graphically in Figure 9. 3 and individual parameters i.e. $D_{10}$, $D_{50}$, $D_{90}$, span value and % droplets having size less than 10 µm are enlisted in Table 9. 3.

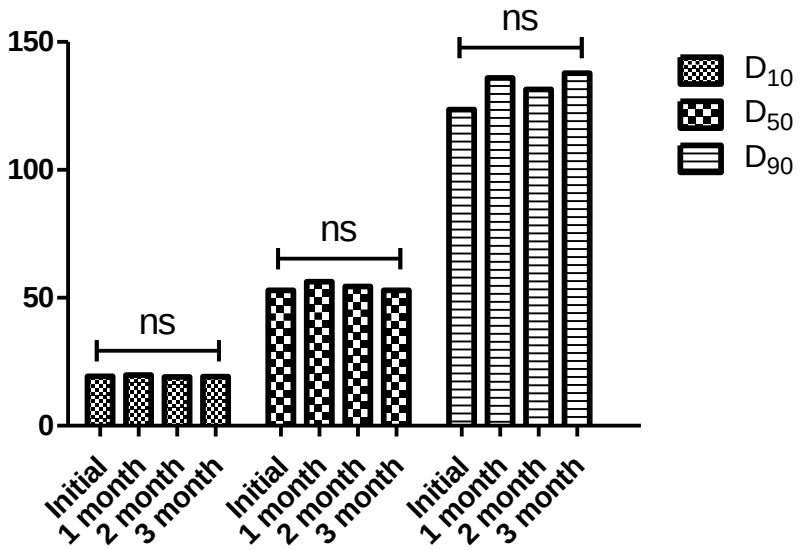


Figure 9. 3: Droplet size distribution of nasal spray at stability time points

Table 9. 3: Droplet size distribution parameters of nasal spray at stability time points

| **Parameters** | **Time points (months)** | | | |
|---|---|---|---|---|
| | **Initial** | **1 month** | **2 months** | **3 months** |
| $D_{10}$ (µm) | 19.28 | 19.87 | 19.02 | 19.14 |
| $D_{50}$ (µm) | 52.95 | 56.37 | 54.41 | 52.92 |
| $D_{90}$ (µm) | 123.54 | 135.90 | 131.46 | 137.76 |
| Span | 1.97 | 2.06 | 2.07 | 2.24 |
| % droplets less than 10 µm | 2.27 | 2.67 | 3.46 | 3.50 |

The data of droplet size distribution showed that there was non-significant change in all the parameters including $D_{10}$, $D_{50}$ and $D_{90}$ and thus supporting uniform viscosity of nasal spray throughout stability period. Additionally, it also verified absence of aggregation and other instability indicating processes and thus proved that the formulated nasal spray would have uniform characteristics throughout stability period.

## 9.4 References

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