 DOI: 10.33892/aph.2021.91.306-307

P-70

Evaluation of a Pneumatic Tube System for Delivering Nivolumab Diluted Solutions

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Keywords: *Monoclonal antibodies, nivolumab, pneumatic delivery, stability*

1. Introduction

Concerns regarding the safety and efficacy of therapeutic proteins have led manufacturers to implement strategies to minimize protein aggregation and particle formation during manufacturing, storage, and shipping. However, once these products are released, manufacturers have limited control over product handling. As an example, pneumatic tube systems (PTS), which are used in hospitals for convenience and reduced turnaround times, are not recommended for active substances that could undergo physical alterations [1]. It has recently demonstrated that in saline polyvinyl chloride bags PTS promoted the extraction of plasticizer nanodroplets which induced the activation of the complement system in an *in vitro* assay [2]. Moreover, diluted solution of rituximab in presence of air into the bag presented signs of aggregation up to 4 passes in PTS, probably due to the air-liquid interface [3].

This work aims to investigate the stability of diluted nivolumab solution after pneumatic delivery, and the effect of residual air inside the bag. Due to the complexity of nivolumab, the major stability-indicating methods were applied based on the results of a previous forced degradation study. All experiments were also carried out after 7 day of storage at 2-8 °C to evidence if the mechanical stress caused by PTS could induce instability over time.

2. Materials And Methods

Under aseptic conditions, nivolumab was diluted by 0.9% sodium chloride in a 100 mL pre-filled polyolefin infusion bag (Baxter VIAFLO®) to obtain the final concentration of 2.4 mg/mL, which corresponds to the recommended flat dose. In another set of bags, air was purged before transportation. Both bags were transported from the Pharmacy compounding department to the Oncologic Day Hospital unit a two-station, bidirectional, SI-TRAIN MC2/160 system. Physico-chemical stability of nivolumab was tested in terms of pH, osmolality, turbidimetry at 350 nm, size exclusion HPLC, NMR and DLS. Moreover, particles concentrations in the IV bags were measured by NTA. All experiments were carried out the same day and after 7 day. Samples not undergoing pneumatic delivery were used as control.

3. Results

Both samples (i.e. with and without air) remained clear for the duration of the study with no precipitates or particulate matter detected with the naked

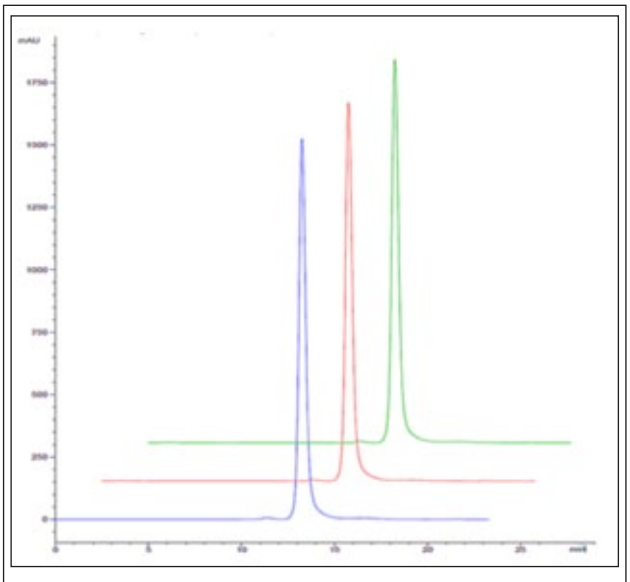

Figure 1 *SE HPLC overlay of diluted nivolumab sample before (blue line) and after transportation in bag with (red line) and without air PN (green line) at day 0.*

eye. The pH of nivolumab solutions ranged from 5.8 to 6.0 over time, which was within the manufacturer's recommended pH range of approximately 6.0 [4]. Also, osmolarity did not undergo to variation ranging from 283 to 297 mOsm/Kg during storage of all samples.

At day 0 diluted samples exhibited chromatograms with two peaks: a single major peak with a retention time of approximately 13 min and a minor signal with a retention time around 11 min. After transportation, samples with and without air presented an identical elution profile, suggesting the absence of soluble aggregate (***Figure 1***). Based on the area values of the two peaks, the percentage of high molecular weight aggregates (HMWP) was calculated to monitor the structural integrity and formation of aggregates during storage time. Despite a light higher percentage of HMWP was detected in samples with air to those without air, this value remained constant upon 7 days. The absence of HMWP aggregates was confirmed by DLS, which is a sensitive and non-perturbating method to evaluate the size of aggregates populations from 1 nm to 6 μm. Indeed, only a monodisperse peak (PDI=0.119) corresponding to a hydrodynamic diameter of about 12 nm was evident in all samples.

Larger aggregates (i.e. >5 μm) were not observed, as demonstrated by turbidimetry experiments. The absorbance at 350 nm slightly increased at day 0 for both samples, probably due to the transport condition, after 7 days the values were close to those of the value of physiological solution.

NMR methodology is extensively applied to study protein stability, since the unfolding leads to a collapse of backbone NH signals around 7 ppm; while the signal intensity decreased in presence of aggregation phenomena. Unfortunately, for nivolumab the large size of the protein and the presence of high amounts of excipients, make possible only the evaluation of the shape and the intensity of the amidic region following the signals of the NH of protein backbone in the region 7-10 ppm. In all samples, the peaks did not collapse toward the chemical shifts associated with random coil structures, suggesting that the chemical environment is not substantially changed after PTS, and the protein seems to remain in its native conformation. Moreover, the amidic region of the three spectra were quite superimposable, without modification of shape and intensity of the main peaks (***Figure 2***). The NMR spectra recorded at day 7 did not display significant peak broadening, nor low signal to noise ratio, typical of aggregated species. Therefore, the overall data suggested that the nivolumab backbone did not undergo to variations and nivolumab maintained its native structure upon 7 days in all the three tested conditions.

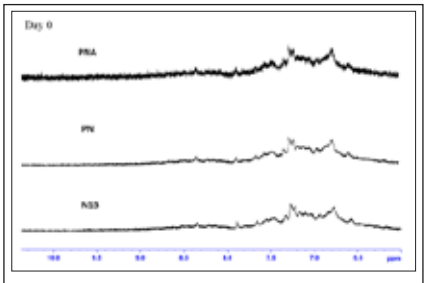

Figure 2 *NMR spectra of the backbone region of nivolumab before (bottom) and after transportation in bag with (up) and (middle) air at day 0.*

NTA revealed a particle level of about $60´10^6$ particles/mL for the physiologic solution used as reference. This value was similar in nivolumab samples with and without air after PTS (day 0) and remained stable upon storage.

4. Conclusions

The assessment of "in-use" stability of biotechnological products is not typically required prior to the regulatory approval. However, "in-use" conditions, *i.e.* handling, storage, transport, could compromise the product stability and, consequently, impact on the safety and/or efficacy of the therapy. In this study, no differences in the main physical and chemical properties were observed in compounded nivolumab solutions after a single pass in PTS for at least 7 days of storage. The presence of air-liquid interface inside the bag was not risk determining for protein stability. In conclusion, these results support the possible use of PTS to deliver bags to clinical services.

References

1. *Wang, P., Nguyen, L. Update to delivering medications via a pneumatic tube system. Am J Health-System Pharm., 74(19): 1521-1522 (2017).*
2. *Snell, J.R., Monticello, C.R., Her, C., Ross, E.L., Frazer-Abel, A.A., Carpenter, J.F., Randolph T.W. DEHP nanodroplets leached from polyvinyl chloride IV bags promote aggregation of IVIG and activate complement in human serum. J. Pharm. Sci., 109: 429-442 (2020).*
3. *Vieillard, K., Rilcy, V., Magneux, C., Bellanger, A., Astier, A., Paul, M. Pneumatic conveying systems and physical stability of monoclonal antibodies: Example of rituximab. Poster presented at: European Society of Oncology Pharmacists (ESOP), Budapest, 2012 Oct 27.*
4. *OPDIVO – EPAR PRODUCT INFORMATION https://www.ema.europa.eu/en/documents/product-information/opdivo-epar-product-information_en.pdf*