PNEUMATIC CONVEYING SYSTEMS AND PHYSICAL STABILITY OF MONOCLONAL ANTIBODIES: THE EXAMPLE OF TRASTUZUMAB

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Introduction

Trastuzumab (TZT) is a monoclonal antibody (mAb) used in the treatment of HER2-positive gastric and breast cancers. Mechanical stress during handling or transportation can induce TZT aggregation, potentially causing loss of efficiency as well as immunogenic adverse effects. In some hospitals, the need of a rapid drug delivery from the pharmacy to the wards has led to use pneumatic systems, but they are currently not used for the transportion of mAb bags due to the paucity of data on possible pitfalls. Nevertheless, some conclusive studies on rituximab and cetuximab have recently been performed and the aim of this work was to determine the influence of pneumatic transportation on the stability of diluted solutions of TZT.

Material and methods

TZT was diluted in NaCl 0,9% to obtain a concentration of 1,2 mg/ml.

One batch of bags (Freeflex® polyolefin bags 50 ml, Fresenius kabi) were prepared at the Department of Pharmacy, Pitié Salpétrière Hospital Group.

Several conditions were tested:

- presence of air (bag 1 to 3) or absence of air (bag 4 to 6).
- travel time
- number of routes (1 to 3)
- All experiments were conducted the same day.

Complementary analytical methods have been used: UV spectroscopy, dynamic light scattering (DLS), describing submicronic populations and their mean diameters, turbidity at 350 nm, size exclusion chromatography (SEC) and fast Fourier transform Infrared spectroscopy (FTIR).

Objectives

The objective of this study was to verify if the pneumatic conveying systems could be used to safely send bags containing the mAb like Trastuzumab to clinical services.

Results

UV spectrophotometry

		279 nm
Bags without air	Bag 1	0.9218 ± 0.0007
	Bag 2	0.9703 ± 0.0002
	Bag 3	0.9807 ± 0.0002
Bags with air	Bag 4	0.9979 ± 0.0004
	Bag 5	0.9904 ± 0.0005
	Bag 6	0.9611 ± 0.0018

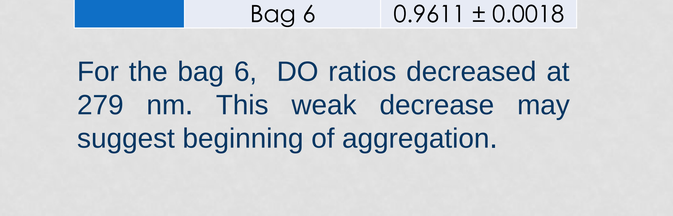
For the bag 6, DO ratios decreased at 279 nm. This weak decrease may suggest beginning of aggregation.

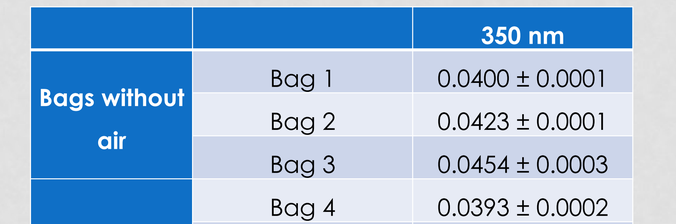
Turbidity

		350 nm
Bags without air	Bag 1	0.0400 ± 0.0001
	Bag 2	0.0423 ± 0.0001
	Bag 3	0.0454 ± 0.0003
Bags with air	Bag 4	0.0393 ± 0.0002
	Bag 5	0.0408 ± 0.0003
	Bag 6	0.0472 ± 0.0001

There is no difference in the turbidity without (0.0424 ± 0.0002) or in the presence of air (0.0426 ± 0.0002). However, bag 6 showed a slight increase, in accordance with its 279 nm ratio decrease

Size exclusion Chromatography

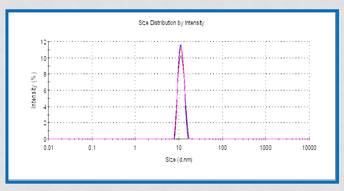


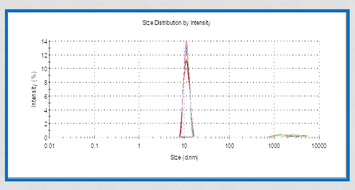


Only one peak was detected by SEC at 15.78 ± 0.01 min with an AUC of 88.37 ± 1.06 mAU.min. No time retention shift or new peak was observed.

Dynamic light scattering

	Pic 1		Pic 2		
	Diameter (nm)	SD	Diameter (nm)	Polydispersity index	Conclusion
Bag 1	11,39	0,0318	0	0,070	Monodisperse
Bag 2	11,36	0,0298	0	0,082	Monodisperse
Bag 3	11,37	0,0149	0	0,076	Monodisperse
Bag 4	11,40	0,0171	0	0,075	Monodisperse
Bag 5	11,36	0,0126	0	0,114	Polydisperse
Bag 6	11,44	0,0090	830,1	0,157	Polydisperse





For bag 1 to 3 DLS revealed a unique population (polydispersity index (PDI) < 0.1) of a mean diameter of 11.38 ± 0.02 nm. For bags with air, a significant increase in polydispersity (PDI=0.157) was observed due to the appearance of a second population by DLS.

FTIR spectroscopy

II° structure	Bag 1	Bag 2	Bag 3	Bag 4	Bag 5	Bag 6
β- Sheet	66.69 ± 0.08	63.60 ± 0.78	58.19 ± 2.22	58.92 ± 0.01	52.18 ± 0.04	49.43± 0.09
β-turn	25.83 ± 0.10	30.25 ± 1.17	28.24 ± 0.78	23.68 ± 0.02	21.54 ± 0.03	28.66± 0.06
Random	7.52 ± 0.06	7.36 ± 0.03	7.67 ± 0.87	17.40 ± 0.02	21.64 ± 0.03	21.43± 0.01
α- Helix	0	0	0	0	0	0

For bag 1 to 3, no secondary structure modifications are apparent by FT-IR. On the other hand, for bag with residual air, significant modifications in the proportion o β-sheets (↓) and random coils (↑), indicating an alteration of the secondary structure.

Discussion - Conclusion

Without air in the bag (purging), no modifications were observed comparatively to the reference (no route) after up to 3 routes. With residual air, some significant changes occurred in bags undergoing 3 routes.

All of these modifications demonstrated destabilization of the TRZ structure and a beginning of aggregation.

In conclusion, aggregation, as a sign of physical instability, is correlated to the presence of hydrophobic air/liquid interfaces, as previoulsy demonstrated with rituximab and cetuximab.

The absence of residual air in the bag (i.e. by purging) is mandatory for the safe use of pneumatic systems to transport mAbs.

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