Pneumatic conveying systems and physical stability of monoclonal antibodies: example of Cetuximab

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Background:

Proteins such as monoclonal antibodies (mAb) are sensitive products which could undergo complex degradation pathways during the various manipulation steps also during transport. Aggregation can be induced by mechanical stresses which can occur during manipulations and transport and could induce loss of efficacy and/or toxic effects such as immunogenicity. Currently pneumatic conveying systems are in place in some hospitals but are not currently used for transport of proteins. Previous studies with Rituximab showed that the use of these systems were possible on the condition of removing air of bags. The objective of this study was to confirm these results with another antibody, Cetuximab.

Material and Method:

Various protein characterization methods were used: size exclusion chromatography (SEC), dynamic light scattering (DLS) describing submicronic populations and corresponding mean diameters, turbidity (350 nm) and infra-red spectroscopy (FTIR) were used to determine changes in physical properties of Cetuximab aggregation mechanically induced. Several conditions were tested: presence of residual air in bags, travel time, number of travel cycles (1 to 3). One concentration was tested (2 mg/ml). All experiments were performed in the same day.

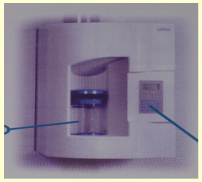
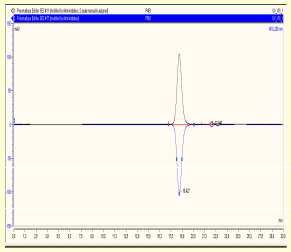


Figure 1: Chromatogram in mirror for SEC

Only one peak in SEC with a retention time of 18.42 ± 0.01 min and an AUC of 62.91 ± 0.75 mAU*min.

A second peak at 22 min was observed for all conditions corresponding to the buffer.



		350 nm
Without air	Bag 1	0,00123 ± 0,00075
	Bag 2	0,00168 ± 0,00031
	Bag 3	0,00216 ± 0,00036
With air	Bag 4	0,00124 ± 0,00040
	Bag 5	0,00229 ± 0,00146
	Bag 6	0,00264 ± 0,00068

Bags 1 and 4: no cycle
Bags 2 and 5: 1 cycle
Bags 3 and 6: 3 cycles

Table 1: Just a slight increase of optical densities (0.00123 to 0.00216) is observed, nevertheless optical densities remained low.

For bags with air, an increase in optical densities was also observed (0.00124 to 0.00264).

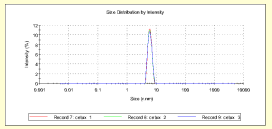


Figure 2: Mean hydrodynamic diameter (nm) for bag 3 (3 cycles withour air)

A monodisperse repartition was observed (polydispersity indexes were inferior to 0.1) with only one population md = 12.56 ± 0.121 nm in DLS.

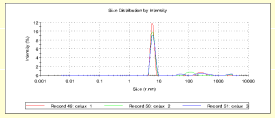


Figure 3: Mean hydrodynamic diameter (nm) for bag 6 (3 cycles with air)

2 populations were found by DLS with a polydispersity index of about 0.22 and with the presence of second populations.

Figure 4: Derivated spectra for bags 2 (pink, 1 cycle, without air) and 5 (red, 1 cycle, with air)

No modification of the FT-IR spectra was observed. Similarity coefficients were close to 1. With or without air, no modification was observed.

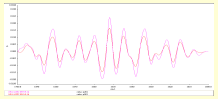
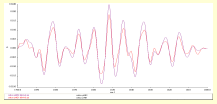


Figure 5: Derivated spectra for bags 4 (purple, 3 cycles, without air) and 6 (red, 3 cycles, with air)

No modification of the FT-IR spectra was observed. Similarity coefficients were close to 1. With air, after 1 or 3 cycles, no modification was observed.



Results and Discussion:

Considering the results obtained with Rituximab, we have limited our experiments to 3 travel cycles. Up to 3 travel cycles and without head space or bubbles into the bags, no modification was noticed in comparison with the control (no run). Indeed, we observed only one peak by SEC with a retention time of 18, 42 ± 0.01 min, a monodisperse population (polydispersity index ≤ to 0.1) with a mean diameter of 12.56 ± 0.121 nm by DLS, a slightly increased of optical densities (OD) at 350 nm (0.00123 up to 0.00216) and no modification of the FT-IR spectra (similarity coefficients were close to one). In the opposite, in presence of air, significant modifications were found after 1 cycle since OD reached to 0.00264 and 2 populations were found by DLS with a polydispersity index of about 0.22. Moreover, modifications of FTIR spectra were also observed (similarity coefficient < 1) suggested alteration of the secondary structure.

Conclusion:

As shown for Rituximab, aggregation of monoclonal antibodies during the pneumatic conveying is strongly dependant on the presence of air/liquid interfaces.