Use of surfactants to avoid aggregation of monoclonal antibodies during the aerosolization process

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CONTEXT & BACKGROUND

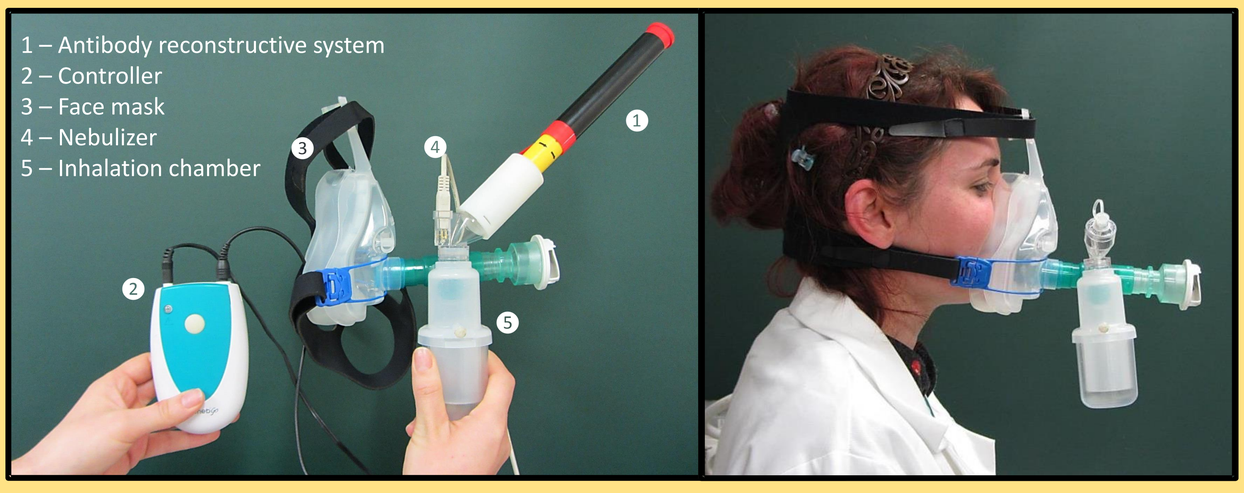
Castor bean also called Ricin is a toxin classified as a priority of biological agents by the French Defense Ministry. Ricin risk is mainly associated to aerosol diffusion. Vaccines showed limited efficacy to induce neutralizing antibody in a pulmonary ricin intoxication (Pincus S.H. *et al.*, 2011), while the local delivery of recombinant neutralizing antibodies raised against the A subunit of ricin (Poli M.A. *et al.*, 1996 ; Guo J. *et al.*, 2006), such as the 43RCA antibody developed by IRBA (Pelat T. *et al.*, 2009) led to animal survival up to 6 hours after intoxication. Thereby the airways represent a promising therapeutic approach and an alternative to the systemic route for the delivery of local-acting monoclonal antibodies (Mabs). Recently, the CEPR (INSERM U1100/EA6305) in Tours, specialized in the aerosol delivery of drugs, has demonstrated the feasibility and the clinical interest of delivering antibodies through the airways as an aerosol to treat lung affections (Maillet A. *et al.*, 2008 ; Maillet A. *et al.*, 2011) and showed that mesh nebulizers are the most reliable devices to efficiently administer liquid formulations of Mabs into the lungs and limit formation of massive insoluble subvisible aggregates.

Ricin: Biological toxin with high potential of bioterrorism

Castor Beans (*Ricinus communis*)

- **[Ricin]** per beans 1 to 10 %
  (ingestion of 3 castor beans = death)
- **Lethal dose in Human through inhalation:** 1 to 10 µg/kg
  (10x lower than ingested ricin)

- **Therapeutic approach proposed:**
  Aerosoltherapy with neutralizing monoclonal antibody (IgG 43RCA, IRBA)
  (Local delivery of 43RCA protected mice against pulmonary intoxication to ricin)
- **Inhibition of 5 DL50 ricin:** 4.5 mg of 43RCA in lung



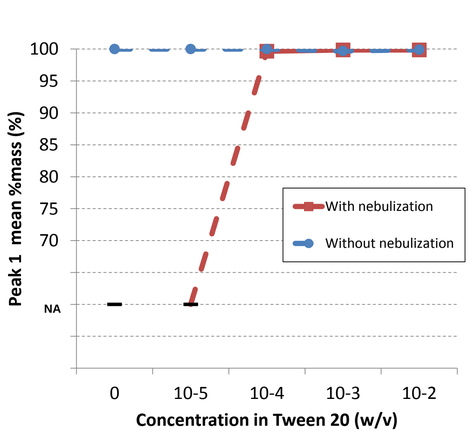
In this study, we evaluated the ability of surfactants, which are usually added to stabilize injectable Mab products, to avoid generation of small size and subvisible aggregates during nebulization of a human IgG.

METHODS & RESULTS

We compared aggregation of a human polyclonal IgG upon mesh-nebulization (Solo, Aerogen, MMAD 4 µm) formulated at 10 mg/mL in PBS 1X in the presence of various concentrations of surfactants (Tween 20, 0 to 10^{-5} % w/v). Dynamic Light Scattering (DLS) and fluorescent microscopy were used to monitor IgG monomers, oligomers (< 1 µm) and larger aggregates (> 2.5 µm) respectively. The viscosity of formulations, flow rate and aerosol volume mean diameter (using a laser diffractometer) were also evaluated.

Formulation IgG and conditions	Peak1, % Mass	Peak1, % Pd	Peak1, Diameter (nm)
No nebulization	99.7-100.0	17.8-25.5	11.2-13.2
Nebulization, Tween 20 0	not available	/	/
Nebulization, Tween 20 10^{-5}	not available	/	/
Nebulization, Tween 20 10^{-4}	99.6-99.9	17.9-25.6	10.9-11.7
Nebulization, Tween 20 10^{-3}	99.7-100.0	22.9-29.6	11.0-11.9
Nebulization, Tween 20 10^{-2}	99.9-100.0	25.8-26.9	10.9-11.4

Figure 1:
- Top and left, IgG monomeric species analyzed by DLS (DynaPro, Wyatt). The acquisition time is set to 10 seconds for a total of 20 acquisitions –*Not available* means that less than 70 % of acquisitions were readable–
  Mass of IgG monomer (min/max % Mass) in solution, polydispersion (min/max %Pd) and size (min/max diameter) of peak 1.
- Right, IgG aggregates analyzed by fluorescent microscopy following staining with red nile.



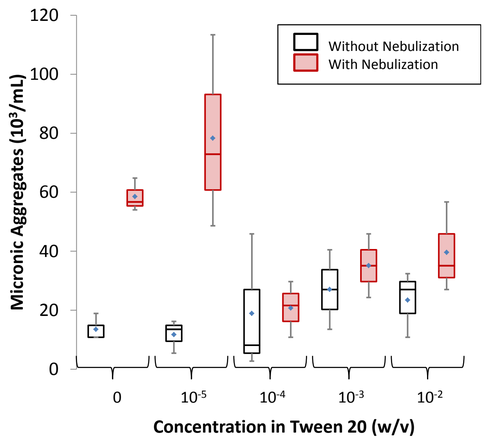
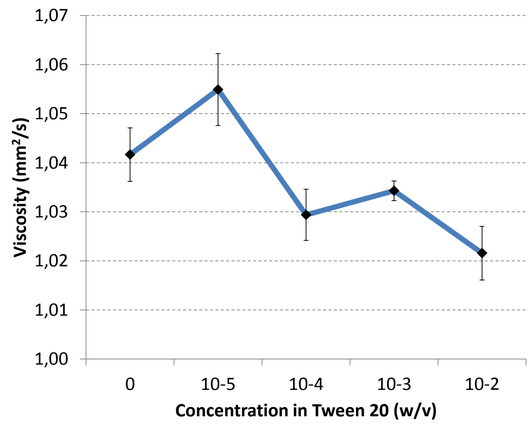
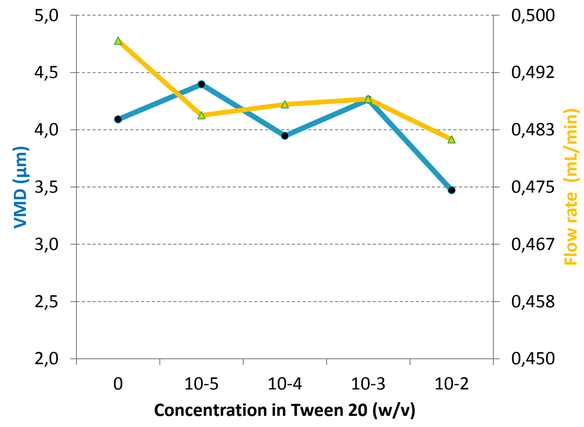


Figure 2: Viscosity, volume median diameter and flow rate were measured for each formulation of IgG (10 mg/mL) containing various amount of Tween 20.





CONCLUSION

Surfactants limited formation of IgG oligomers and larger aggregates during the nebulization process. Moreover, flow rate, VMD and viscosity remained unaffected by surfactant concentration (up to 10^{-2} %). In conclusion, surfactants may be used to stabilize IgG during aerosolization. However, considering the lack of consistent data on the safety of surfactants delivered through the pulmonary route, levels of surfactants would rather be maintained as low as possible.

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